PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 15/00, 15/28, C12P 21/02 C07H 15/12, C12Q 1/68 G01N 22/53	A1	**(11) International Publication Number:** WO 92/12176 **(43) International Publication Date:** 23 July 1992 (23.07.92)

(21) International Application Number: PCT/US92/00337

(22) International Filing Date: 14 January 1992 (14.01.92)

(30) Priority data:
640,492 14 January 1991 (14.01.91) US

(71) Applicant: NEW YORK UNIVERSITY [US/US]; 550 First Avenue, Room MSB 153, New York, NY 10016 (US).

(72) Inventors: LEE, Tae, Ho ; 206 Pleasant View Drive, Piscatawa, NJ 08855 (US). LEE, Gene, W. ; 23 Lexington Avenue, New York, NY 10021 (US). VILCEK, Jan ; 180 E. 79th Street, New York, NY 10021 (US).

(74) Agent: BROWDY, Roger, L.; Browdy and Neimark, 419 Seventh Street, Ste. 300, N.W., Washington, DC 20004 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CYTOKINE-INDUCED PROTEIN, TSG-14, DNA CODING THEREFOR AND USES THEREOF

(57) Abstract

Pleiotropic pro-inflammatory cytokines, such as TNF and IL-1, induce expression of a protein molecule, termed TSG-14, in connective tissue cells. The TSG-14 protein and functional derivatives thereof, DNA coding therefor, expression vehicles, such as a plasmid, and host cells transformed or transfected with the DNA molecule, and methods for producing the protein and the DNA are provided. Antibodies specific for the TSG-14 protein are disclosed, as is a method for detecting the presence of TSG-14 protein in a biological sample, using the antibody or another molecule capable of binding to TSG-14 such as hyaluronic acid. A method for detecting the presence of nucleic acid encoding a normal or mutant TSG-14 protein, a method for measuring induction of expression of TSG-14 in a cell using either nucleic acid hybridization or immunoassay, a method for identifying a compound capable of inducing the expression of TSG-14 in a cell, and a method for measuring the ability of a cell to respond to TNF are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MN**	Mongolia
BE	Belgium	**GA**	Gabon	**MR**	Mauritania
BF	Burkina Faso	**GB**	United Kingdom	**MW**	Malawi
BG	Bulgaria	**GN**	Guinea	**NL**	Netherlands
BJ	Benin	**GR**	Greece	**NO**	Norway
BR	Brazil	**HU**	Hungary	**PL**	Poland
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**RU**	Russian Federation
CG	Congo	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CH	Switzerland			**SE**	Sweden
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SN**	Senegal
CM	Cameroon	**LI**	Liechtenstein	**SU**	Soviet Union
CS	Czechoslovakia	**LK**	Sri Lanka	**TD**	Chad
DE	Germany	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America

CYTOKINE-INDUCED PROTEIN, TSG-14, DNA CODING THEREFOR AND USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to a protein, TSG-14, inducible in connective tissue cells by tumor necrosis factor or interleukin-1, DNA and mRNA encoding the TSG-14 protein, functional derivatives of the protein, antibodies specific to the protein, methods of producing the protein and DNA, and uses of the protein, DNA, mRNA, peptides and antibodies.

Description of the Background Art

Tumor necrosis factor (TNF) is a powerful pleiotropic cytokine important in host defenses against tumors and infectious agents. TNF has also been implicated in the pathology of some neoplastic diseases, infections and autoimmune disorders. Most biological actions of TNF can be attributed to the triggering of complex genetic programs in the target cells. Several genes activated by TNF have been identified but many more require characterization.

General Properties of TNF

TNF (also termed TNF-α and cachectin) is a protein produced by activated monocytes/macrophages which was originally detected in the serum of animals injected sequentially with a bacterial vaccine (bacillus Calmette-Guerin, BCG) and endotoxin (Carswell, E.A. et al., Proc. Natl. Acad. Sci. USA 72:3666 (1975)). TNF is structurally and functionally related to a cytokine produced by activated T

lymphocytes which was originally termed lymphotoxin (LT) and is also known as TNF-ß (Aggarwal, B.B. et al., J. Biol. Chem. 260:2334 (1985); Williams, T.W. et al., Nature 219:1076 (1968); Ruddle, N.H. et al., J. Exp. Med. 128:1267 (1968); Spies, T. et al., Proc. Natl. Acad. Sci. USA 83:8699 (1986); Gray, P.W. et al., Nature 312:721 (1984); Pennica, D.W. et al., Nature 312:724 (1984)). The genes encoding TNF and LT are linked, and are near the HLA-DR locus on the short arm of human chromosome 6 (Spies, T. et al., supra). TNF and LT bind to common cell surface receptors (Aggarwal, B.B. et al., Nature 318:665 (1985)).

Natural human TNF is a 157 amino acid, non-glycosylated protein with a molecular weight of approximately 17 kDa under denaturing conditions. The mature molecule is derived from a precursor (pre-TNF) which contains 76 additional amino acids at the N-terminus (Pennica, D.W. et al., supra). The expression of the gene encoding TNF is not limited to cells of the monocyte/macrophage family. Several human non-monocytic tumor cell lines were shown to produce TNF (Rubin, B.Y. et al., J. Exp. Med. 164:1350 (1986); Spriggs, D. et al., Proc. Natl. Acad. Sci. USA 84:6563 (1987)). TNF is also produced by $CD4^+$ and $CD8^+$ peripheral blood T lymphocytes, and by various cultured T and B cell lines (Cuturi, M.C., et al., J. Exp. Med. 165:1581 (1987); Sung, S.-S.J. et al., J. Exp. Med. 168:1539 (1988)).

Accumulating evidence indicates that TNF is a regulatory cytokine with pleiotropic biological activities. These activities include: inhibition of lipoprotein lipase synthesis ("cachectin" activity) (Beutler, B. et al., Nature

316:552 (1985)), activation of polymorphonuclear leukocytes (Klebanoff, S.J. et al., J. Immunol. 136:4220 (1986); Perussia, B. ,et al., J. Immunol. 138:765 (1987)), inhibition of cell growth or stimulation of cell growth (Vilcek, J. et al., J. Exp. Med. 163:632 (1986); Sugarman, B.J. et al., Science 230:943 (1985); Lachman, L.B. et al., J. Immunol. 138:2913 (1987)), cytotoxic action on certain transformed cell types (Lachman, L.B. et al., supra; Darzynkiewicz, Z. et al., Canc. Res. 44:83 (1984)), antiviral activity (Kohase, M. et al., Cell 45:659 (1986); Wong, G.H.W. et al., Nature 323:819 (1986)), stimulation of bone resorption (Bertolini, D.R. et al., Nature 319:516 (1986); Saklatvala, J., Nature 322:547 (1986)), stimulation of collagenase and prostaglandin E2 production (Dayer, J.-M. et al., J. Exp. Med. 162:2163 (1985)), and other actions. For reviews of TNF, see Beutler, B. et al.. Nature 320:584 (1986); Old, L.J., Science 230:630 (1986); and Le, J. et al., Lab. Invest. 56:234 (1987).

TNF also has immunoregulatory actions, including activation of T cells (Yokota, S. et al., J. Immunol. 140:531 (1988)), B cells (Kehrl, J.H. et al., J. Exp. Med. 166:786 (1987)), monocytes (Philip, R. et al., Nature 323:86 (1986)), thymocytes (Ranges, G.E. et al., J. Exp. Med. 167:1472 (1988)), and stimulation of the cell-surface expression of major histocompatibility complex (MHC) class I and class II molecules (Collins, T. et al., Proc. Natl. Acad. Sci. USA 83:446 (1986); Pujol-Borrell, R. et al., Nature 326:304 (1987)).

TNF also has various pro-inflammatory actions which result in tissue injury, such as induction of procoagulant

activity on vascular endothelial cells (Pober, J.S. et al., J. Immunol. 136, 1680, 1986)), increased adherence of neutrophils and lymphocytes (Pober, J.S. et al., J. Immunol. 138:3319 (1987)), and stimulation of the release of platelet activating factor (PAF) from macrophages, neutrophils and vascular endothelial cells (Camussi, G. et al., J. Exp. Med. 166:1390 (1987)). Recent evidence implicates TNF in the pathogenesis of many infections (Cerami, A. et al., Immunol. Today 9:28 (1988)), immune disorders (Piguet, P.-F. et al., J. Exp. Med. 166:1280 (1987)), and in cachexia accompanying some malignancies (Oliff, A. et al., Cell 50:555 (1987)). Michie, H.R. et al., Br. J. Surg. 76:670-671 (1989), reviewed evidence that TNF is the principal mediator associated with the pathological changes of severe sepsis.

TNF also has activity associated with growth and differentiation of hemopoietic precursor cells (Murphy, M. et al., J. Exp. Med. 164:263 (1986); Broxmeyer, H.E. et al., J. Immunol. 136:4487 (1986)); some of these actions may be indirect, and are thought to be mediated through the stimulation of production of granulocyte-macrophage colony stimulating factor (GM-CSF) (Munker, R. et al., Nature 323:79 (1986)) and other hemopoietic growth factors (Zucali, J.R. et al., J. Immunol. 140:840 (1988)).

Regulation of Gene Expression by TNF

It is, therefore, apparent that TNF is an extremely "versatile" and clinically significant cytokine. Most of its actions are likely to be mediated by the activation or inactivation of specific genes in the cells upon which it acts. One exception to this mode of action is the rapid

cytotoxic effect of TNF on certain target cells; this effect is augmented by inhibitors of RNA or protein synthesis and does not appear to depend on the modulation of gene expression (Matthews, N., Br. J. Cancer 48:405 (1983)). Many specific gene products have been shown to be up-regulated in TNF-treated cells, some of which are discussed below.

Among the first examples of TNF-modulated gene expression was the demonstration that TNF treatment induced an increase in MHC class I mRNA levels and in surface expression of the MHC class I glycoproteins in human vascular endothelial cells (HUVEC) and normal skin fibroblasts (Collins, T. et al., supra). A partial list of other molecules (or genes) induced by TNF appears in Table 1, below. It is interesting to note that TNF is an autoregulatory cytokine, since exogenously added TNF increases TNF synthesis in monocytes and monocytic cell lines (Philip, R. et al., Nature 323:86 (1986); Schmid, J. et al., J. Immunol. 139:250 (1987)).

Table 1

GENES AND PROTEINS INDUCED BY TUMOR NECROSIS FACTOR

Protein or Gene	Cell Type	Ref
Leukocyte adhesion protein H4/18	HUVEC	(1)
Platelet-derived growth factor (PDGF)	HUVEC and some tumor cell lines	(2)
IL-6 (IFN-β2 or BSF-2)	Human skin fibroblasts	(3)
HLA-DR	Human tumor cell lines	(4)
Collagenase	Synovial cells and skin fibroblasts	(5)
2'-5' oligoadenylate synthetase	Tumor cell lines	(6)
c-myc and c-fos oncogenes	Human skin fibroblasts	(7)
Epidermal growth factor receptor	Human skin fibroblasts	(8)
Tissue factor	HUVEC	(9)
ICAM-1 and ELAM-1	HUVEC	(10)
Plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2)	HT1080 cell line	(11)
Synthesis of 36 kDa and 42 kDa (=PAI-2) proteins	Human skin fibroblasts	(12)
Superoxide Dismutase (MnSOD) gene	Human tumor cell lines	(13)
IL-1α and IL-1β genes	Human skin fibroblasts	(14)

REFERENCES: 1. Pober, J.S. et al., J. Immunol. 136, 1680, 1986. 2. Hajjar, K.A. et al., J. Exp. Med. 166, 235, 1987. 3. Kohase, M. et al., Cell 45:659 (1986). 4. Pfizenmaier, K. et al. J. Immunol. 138, 975, 1987. 5. Dayer, J.-M. et al., J. Exp. Med. 162:2163 (1985). 6. Wong, G.H.W. et al., Nature 323:819 (1986). 7. Lin, J.-X. et al., J. Biol. Chem. 262, 11908, 1987. 8. Palombella, V.J. et al., J. Biol. Chem. 262, 1950, 1987. 9. Edgington, T.S. et al., Abs. 2ndInternat. Conf. TNF, p. 4, 1989. 10. Bevilacqua, M.P. et al., Proc. Natl. Acad. Sci. USA 84, 9238, 1987. 11. Medcalfe, R.L. et al., J. Exp. Med. 168, 751, 1988. 12. Kirstein, M. et al., J. Biol. Chem. 261, 9565, 1986. 13. Wong, G.H. et al., Science 242, 941, 1988. 14. Le, J. et al. Lab. Invest. 56:234 (1987).

The inhibitory actions of TNF on gene expression are less well-characterized. TNF was shown to inhibit c-myc expression in cells whose growth it inhibited (Kronke, M. et al., Proc. Natl. Acad. Sci. USA 84:469 (1987)). Collagen synthesis was inhibited in human fibroblasts (Solis-Herruzo et al., J. Biol. Chem. 263:5841 (1988)), and thrombomodulin in HUVEC (Conway, E.M. et al., Molec. Cell. Biol. 8:5588 (1988)). All these inhibitory actions were expressed at the level of transcription, but the precise mechanisms are still unclear.

The mechanisms of signal transduction and gene activation by TNF are the subject of great interest. In many cell types, TNF activates a phospholipase (most likely PLA2), resulting in the liberation of arachidonic acid from cellular pools (Suffys, P. et al., Biochem. Biophys. Res. Comm. 149:735 (1987)) and increased eicosanoid synthesis (Dayer, J.-M. et al., supra). In human fibroblasts, TNF stimulated GTPase activity (Imamura, K. et al.J. Biol. Chem. 263:10247 (1989)), raised cAMP levels, enhanced cAMP-dependent protein kinase activity, and activated protein kinase C (PKC) (Zhang, Y. et al., Proc. Natl. Acad. Sci. USA 85:6802 (1988); Brenner, D.A. et al., Nature 337:661 (1989)). TNF can also activate the transcription factor NF-kB, which appears to be the mechanism by which TNF induces the IL-2 receptor α chain (Lowenthal, J.W. et al., Proc. Natl. Acad. Sci. USA 86:2231 (1989)) or cause activation of latent human immunodeficiency virus, HIV-1 (Griffin, G.E. et al., Nature 339:70 (1989)).

Interactions of TNF with other Cytokines

When the individual actions of TNF-α, TNF-ß, IL-1α, IL-1ß, IFN-α, IFN-ß or IFN-gamma are compared in various experimental systems, a great deal of apparent redundancy and ambiguity is noted. First, structurally related cytokines which utilize the same receptor (e.g., TNF-α and TNF-ß; IL-1α and IL-1ß; IFN-α and IFN-ß) act similarly. More surprisingly, structurally unrelated cytokines which bind to different receptors also have similar physiological effects. For example, IL-1 and TNF have similar gene activating activities, and result in similar biological effects (Le, J. et al. Lab. Invest. 56:234 (1987)). IFNs and TNF also share biological

activities (Kohase, M. et al., Cell 45:659 (1986); Wong, G.H.W. et al., Nature 323:819 (1986); Williamson, B.D. et al., Proc. Natl. Acad. Sci. USA 80:5397 (1983); Stone-Wolff, D.S. et al., J. Exp. Med. 159:828 (1984)). For example, IFNs and TNF activate some of the same genes, including MHC class I and class II genes, 2'-5' oligo-adenylate synthetase, IL-6, the transcription factor IRF-1, and the TNF gene itself (Vilcek, J., Handbook of Experimental Pharmacology, Vol. 95/II, p. 3, Springer-Verlag, Berlin (1990)).

Under natural conditions cells are rarely, if ever, exposed to a single cytokine. Rather, cytokine action in vivo is "contextual," as has been postulated for growth factors (Sporn, M.B. et al., Nature 332:217 (1988)). The biological effects produced by cytokines under natural conditions must therefore represent the sum of the synergistic and antagonistic interactions of all cytokines present simultaneously in a given microenvironment. In addition, cytokines appear to be arranged in "networks" and "cascades", such that the synthesis of one cytokine can be positively or negatively regulated by another. For these reasons, it is important to understand the molecular mechanisms of action of cytokines acting individually as well as in combination.

In contrast to the above, there are cases in which the actions of TNF and IFNs are antagonistic rather than similar or synergistic. For example, TNF is mitogenic for human diploid fibroblasts, whereas IFNs inhibit growth of these cells (Vilcek, J. et al., J. Exp. Med. 163:632 (1986)). The cellular response to a combination of TNF and an IFN can differ from the response to either one alone, both qualitatively and quantitatively (Leeuwenberg, J.F.M. et al., J. Exp. Med. 166:1180 (1987); Reis, L.F.L. et al., J. Biol. Chem. 264:16351 (1989); Feinman, R. et al., J. Immunol. 136:2441 (1986); Trinchieri, G. et al., Abstr. 2nd Int'l Conf. TNF, p. 7 (1989)). To make matters even more complicated, in some cells TNF can induce IFN-ß synthesis (Reis et al., supra); the activation of some genes (e.g., HLA class I) by TNF requires the presence of IFN-ß (Leeuwenberg et al., supra). Since IFNs and TNF-α and TNF-ß are often produced in

the same microenvironment in response to a similar set of stimuli (Murphy, M. et al., supra; Stone-Wolff et al., supra; Billiau, A., Immunol. Today 9:37 (1988)), it is clear that the interactions of TNF and IFNs are highly relevant to the outcome in vivo under either "normal" or pathophysiological conditions.

The association of cytokines, in particular TNF, with cancer and infectious diseases takes many forms often related to the host's catabolic state. One of the major and most characteristic problems seen in cancer patients is weight loss, usually associated with anorexia. The extensive wasting which results is known as "cachexia" (see, for review, Kern, K.A. et al. (J. Parent. Enter. Nutr. 12:286-298 (1988)). Cachexia includes progressive weight loss, anorexia, and persistent erosion of body mass in response to a malignant growth. The fundamental physiological derangement may be related to a decline in food intake relative to energy expenditure. The causes for this commonly observed and often life-limiting disturbance remain to be determined, even though many contributing factors have been identified (Braunwald, E. et al. (Eds.), Harrison's PRINCIPLES OF INTERNAL MEDICINE, 11th Ed., McGraw-Hill Book Co., New York, 1987, Chap. 78, pp. 421-431). The cachectic state is associated with significant morbidity and is responsible for the majority of cancer mortality. A number of studies have suggested that TNF is an important mediator of the cachexia in cancer, infectious disease, and in other catabolic states.

It has been known for some time that in bacterial infection, sepsis and critical illness, bacterial lipopolysaccharides (LPS), or endotoxins, are responsible for many of the pathophysiological manifestations, including fever, malaise, anorexia, and cachexia. More recently, it was observed that TNF can mimic many endotoxin effects, leading to the suggestion that TNF, and related cytokines derived from cells of the macrophage/monocyte family, in particular, IL-1, are central mediators responsible for the clinical manifestations of the illness. Endotoxin is a potent monocyte/macrophage activator which stimulates production and

secretion of TNF (Kornbluth, S.K. et al., J. Immunol. 137:2585-2591 (1986)) and other cytokines including IL-1 (Dinarello, C.A., Rev. Infec. Dis. 6:51-94 (1984)), interleukin-6 (IL6), and colony stimulating factor (CSF) (Apte, R.N. et al. J. Cell. Physiol. 89:313 (1976)). Some of these cytokines further stimulate T lymphocytes to produce additional cytokines, for example, interleukin-2 (IL-2) (Robb, R.J., Immunol. Today 5:203-209 (1984)).

The monocyte-derived cytokines are thought to be important mediators of the metabolic and neurohormonal responses to endotoxin (Michie, H.R. et al., N. Eng. J. Med. 318:1481-1486 (1988)), and in cancer and other catabolic states (Norton, J.A. et al., Nutrition 5:131-135 (1989)). Interestingly, some changes induced by low-dose TNF closely resemble changes provoked by high dose IL2 (Remick, D.G. et al., Lab. Invest. 56:583-590 (1987)).

Endotoxin administration to human volunteers produced acute illness with flu-like symptoms including fever, tachycardia, increased metabolic rate and stress hormone release (Revhaug, A. et al., Arch. Surg. 123:162-170 (1988)). Treatment of cancer patients (having normal kidney and liver function) with escalating doses of TNF (4 - 636 $\mu g/m^2/24$ hr) indicated that doses greater than 545 $\mu g/m^2/24$hr caused alterations similar to those induced by injection of endotoxin (4 ng/kg) into healthy humans (Michie, H.R. et al., Surgery 104:280-286 (1988)), leading the authors to conclude that TNF is the principal host mediator of septic and endotoxemic responses. More recently, it was shown that five days of chronic intravenous TNF infusion into humans or rats was associated with anorexia, fluid retention, acute phase responses, and negative nitrogen balance (i.e., classic catabolic effects), leading to the conclusion that TNF may be responsible for many of the changes noted during critical illness (Michie, H.R. et al., Ann. Surg. 209:19-24 (1989)). Administration of rTNF to cancer patients also led to a rise in C-reactive protein (CRP) and a fall in serum zinc, a large increase in forearm efflux of total amino acids, and amino acid uptake by other tissues (Warren, R.S. et al., Arch.

Surg. 122:1396-1400 (1987)), considered further evidence for a role of TNF in cancer cachexia.

SUMMARY OF THE INVENTION

Cytokines such as TNF and IL-1 play a major role in the mediation of inflammatory responses as well as in host responses to infections and cancer. The mode of action of these cytokines is only beginning to be understood. The present inventors have discovered and studied a series of proteins and glycoproteins induced in connective tissue cells by such cytokines. As a result of these studies, the present inventors have conceived of the use of such cytokine-induced proteins or glycoproteins, termed TSG proteins, or functional derivatives such as peptides derived therefrom, and antibodies specific for these TSG proteins/glycoproteins, for a number of diagnostic and therapeutic procedures. These proteins, the DNA coding therefor, and the functional derivatives thereof, are useful in a number of diseases associated with action of the above types of cytokines, including chronic inflammatory conditions, in particular rheumatoid arthritis, in infections and sepsis, and in cancer.

Specifically, the present invention provides a cytokine-induced protein or glycoprotein molecule, termed TSG-14, or a functional derivative thereof, wherein, when the protein molecule is one which naturally occurs, it is substantially free of other proteins or glycoproteins with which it is natively associated. The full length protein molecule has an apparent molecular weight of about 19.5 kDa, including the probable signal sequence; the molecular weight of the mature protein is about 17.5 kDa. The TSG-14 protein has the amino acid sequence SEQ ID NO:2.

The present invention is further directed to a DNA molecule encoding TSG-14 or a functional derivative thereof, wherein when the DNA molecule occurs naturally, it is substantially free of other nucleotide sequences with which it is natively associated. In a preferred embodiment, the DNA molecule has the nucleotide sequence SEQ ID NO:1. The DNA

molecule of the present invention may be genomic DNA or cDNA, and it may be single stranded or double stranded.

The present invention provides the DNA molecule as an expression vehicle, such as a plasmid, and provides host cells transformed or transfected with the DNA molecule. Hosts may be bacteria or eukaryotic cells, including yeast and mammalian cells.

Also included in the present invention is a process for preparing the TSG-14 protein or glycoprotein molecule substantially free of other proteins or glycoproteins with which it is natively associated, or a functional derivative thereof, comprising: (a) culturing a host cell capable of expressing the protein under culturing conditions, (b) expressing the protein or functional derivative; and (c) recovering the protein or functional derivative from the culture.

The present invention is also directed to an antibody specific for the TSG-14 protein or an epitope thereof. A preferred antibody is a monoclonal antibody.

Also provided is a method for detecting the presence of TSG-14 protein in a biological sample, comprising: (a) contacting the biological sample that is suspected of containing TSG-14 protein with a molecule capable of binding to the protein; and (b) detecting any of this molecule bound to the protein. For this method, a preferred molecule is an antibody or antibody fragment, most preferably a monoclonal antibody, and the preferred detection method is an immunoassay.

The present invention further includes a method for detecting the presence of nucleic acid encoding a normal or mutant TSG-14 protein in a subject comprising: (a) contacting a cell obtained from the subject, an extract thereof, or a culture supernatant thereof, with an oligonucleotide probe encoding at least a portion of the normal or mutant TSG-14 under hybridizing conditions; and (b) measuring the hybridization of this probe to the nucleic acid of the cell, thereby detecting the presence of the nucleic acid. This method may additionally include, before step (a), selectively

amplifying the amount of DNA of the cell encoding the TSG-14 protein.

The present invention is still further directed to a method for measuring induction of expression of TSG-14 in a cell, comprising: (a) contacting the cell with a substance capable of inducing expression of TSG-14; (b) measuring the amount of mRNA encoding TSG-14 in the cell by hybridization with an oligonucleotide probe encoding at least a portion of TSG-14, under hybridizing conditions; and (c) comparing the amount of TSG-14 mRNA in the cell with the amount of TSG-14 mRNA in the cell not contacted with the inducing substance, wherein an increase in the amount of the TSG-14 mRNA indicates that the induction has occurred.

An alternative method for measuring induction of expression of TSG-14, according to the present invention comprises: (a) contacting the cell with a substance capable of inducing expression of TSG-14; (b) measuring the amount of TSG-14 protein in an extract or supernatant of the cell using the method described above for measuring the TSG-14 protein, preferably, an immunoassay; and (c) comparing the amount of TSG-14 protein in the cell extract or supernatant with the amount of TSG-14 protein in the extract or supernatant of a cell not contacted with the inducing substance, wherein an increase in the amount of the TSG-14 protein indicates that the induction has occurred.

The present invention may also be used in a method for identifying a compound capable of inducing the expression of TSG-14 in a cell, comprising: (a) contacting the cell with the compound being tested; and (b) measuring the induction of TSG-14 mRNA according to one of the two methods described above,
thereby identifying the compound.

The present invention also provides a method for measuring the ability of a cell to respond to TNF or to IL-1, comprising: (a) contacting the cell with an amount of TNF capable of inducing expression of the TSG-14 gene in FS-4 cells; and (b) determining the induction of expression of TSG-14 mRNA or protein using either of the methods described

above, thereby measuring the ability of the cell to respond to TNF.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts Northern blots showing induction of 5 mRNAs corresponding to eight TSG cDNAs in FS-4 cells treated with TNF. Growth-arrested FS-4 cells were exposed to TNF (20 ng/ml) at 0 h. At different intervals thereafter, total cell RNA was isolated, fractionated on formaldehyde-agarose gels, transferred to Zeta-probe blotting membranes, and hybridized separately to each of the 32p-labeled TSG cDNA inserts. To ascertain whether equal amounts of RNA were loaded in each lane, most blots were also probed with a 32p-labeled pHe7 internal reference cDNA insert specific for an invariant mRNA species of about 1.0 kb.

Figure 2 is a series of graphs showing the kinetics of induction of eight TSG mRNAs by TNF. Autoradiograms of the Northern blots shown in Figure 1 were scanned by laser densitometry. For each individual mRNA, the highest-density band was normalized to represent 100% induction.

Figure 3 is a northern blot and graph (taken from the appropriate portion of Figures 1 and 2) showing the induction of TSG-14 mRNA by TNF. Quiescent FS-4 cells were exposed to 20 ng/ml TNF at 0 hrs. Total cellular mRNA was removed at various time points and was fractionated on a formaldehyde/agarose gel, transferred to a Zeta-probe blotting membrane and hybridized with a 32p-labeled TSG-14 cDNA probe. The autoradiogram was then scanned by laser densitometry an plotted, normalizing the highest intensity band as 100% induction.

Figure 4 shows the cDNA and amino acid sequence of TSG-14. THe hydrophobic signal sequence and the polyadenylation signals are underlined.

Figure 5 is a hydrophobicity plot of the TSG-14 protein. The hydrophobicity was plotted using the DNA Strider program on a Macintosh SE computer, utilizing the algorithm of Kyte and Doolittle (J. Mol. Biol. 157:105 (1982)).

Figure 6 is an acid/base plot of the TSG-14 protein. The isoelectric points of the amino acid residues comprising TSG-14 were calculated and plotted using the DNA Strider program on a Macintosh SE computer.

Figure 7 depicts the pATH2-TSG-14 expression vector. The TSG-14 insert contains a Sau3A site at its 5' end. A Sau3A site at the 3' end of the insert was generated when an EcoRI fragment of TSG-14 was inserted into the pTZ19 vector system, and subsequently cut with the Sau3A enzyme. This insert was then ligated into a unique BamHI site within the coding region of the trpE protein of the pATH2 vector.

Figure 8 is a gel pattern showing expression of the TrpE/TSG-14 fusion protein. E. coli JM101 cells were transformed with the pATH2-TSG-14 expression vector and induced with indole acrylic acid. Whole cell lysates were denatured by boiling in a buffer containing 2% SDS and ß-mercaptoethanol. The denatured samples were electroeluted on a 10% acrylamide gel containing SDS and stained with Coomassie Blue. Lane 1: MW markers; lane 2: control (TrpE only); lanes 3 and 4; truncated trpE-TSG-14 fusion protein - the pATH2-TSG-14 vector was cut with PstI to generate a smaller expressed protein (MW=44 kDa); lanes 5 and 6; TrpE/TSG-14 fusion protein (MW is about 60 kDa).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A number of genes activated in human FS-4 fibroblasts by tumor necrosis factor (TNF) were termed by the present inventors "TNF-stimulated genes" (abbreviated TSG). It should be appreciated that such genes, and the proteins and glycoproteins they encode, are induced by cytokines more generally, including TNF and IL-1. The proteins, functional derivatives, such as peptide fragments, and antibodies to the proteins are useful in a number of methods of importance to the diagnosis and treatment of diseases and conditions in which the activity, or inactivity, of such cytokines is associated with the pathophysiology. Such diseases include chronic inflammation, such as rheumatoid arthritis, cancer, and infections, in particular with gram-negative bacteria.

The present invention is directed to one of these genes and its protein product, both termed TSG-14. The present invention provides TSG-14 DNA, mRNA and protein in substantially pure form, functional derivatives of the protein such as peptide fragments, antibodies specific for the protein, methods of producing the DNA, mRNA and protein, methods of using these molecules.

By "substantially pure" is meant any protein or peptide of the present invention, or any DNA or mRNA sequence encoding any such protein or peptide, which is essentially free of other proteins, DNA sequences or mRNA sequences, respectively, or of other contaminants with which it might normally be found in nature, and, as such, exists in a form not found in nature.

"Substantially free of other proteins" indicates that the protein has been purified away from at least 90 per cent (on a weight basis), and from even at least 99 per cent, if desired, of other proteins and glycoproteins with which it is natively associated, and is therefore substantially free of them. That can be achieved by subjecting the cells, tissue or fluids expressing or containing the TSG-14 protein to protein purification techniques such as immunoadsorbent columns bearing antibodies, such as monoclonal antibodies (mAb) reactive against the protein. Alternatively, the purification can be achieved by a combination of standard methods, such as ammonium sulfate precipitation, molecular sieve chromatography, and ion exchange chromatography.

The methods of the present invention are used to identify normal or mutant TSG-14 genes or measure the presence or amount of TSG-14 protein associated with a cell or tissue, or secreted by a cell; such methods can serve as methods for identifying susceptibility to inflammatory conditions and to sepsis following gram-negative bacterial infections.

In one embodiment, the invention is directed to a naturally occurring TSG-14 protein or glycoprotein substantially free from impurities of human origin with which it is natively associated. In another embodiment, the

invention is directed to a recombinant TSG-14 encoded protein or glycoprotein.

It will be understood that the TSG-14 protein of the present invention can be purified biochemically or physicochemically from a variety of cell or tissue sources. For preparation of naturally occurring TSG-14 protein, connective tissue cells such as human fibroblasts are preferred. Alternatively, methods are well known for the synthesis of polypeptides of desired sequence on solid phase supports and their subsequent separation from the support.

Because the TSG-14 gene can be isolated or synthesized, the TSG-14 polypeptide, or a functional derivative thereof, can be synthesized substantially free of other proteins or glycoproteins of mammalian origin in a prokaryotic organism or in a non-mammalian eukaryotic organism, if desired. As intended by the present invention, a TSG-14 protein or glycoprotein molecule produced by recombinant means in mammalian cells, such as transfected GM637 cells, for example, is either a naturally occurring protein sequence or a functional derivative thereof. Where a naturally occurring protein or glycoprotein is produced by recombinant means, it is provided substantially free of the other proteins and glycoproteins with which it is natively associated.

A preferred use of this invention is the production by chemical synthesis or recombinant DNA technology of fragments of the TSG-14 molecule, which still retain biological activity such as binding to antibodies, and the like. Among the advantages of shorter peptides for some of the methods of the present invention are (1) greater stability and diffusibility, and (2) less immunogenicity. As discussed herein, the TSG-14 proteins or peptides of the present invention may be further modified for purposes of drug design, such as, for example, to reduce immunogenicity, to promote solubility or enhance delivery, or to prevent clearance or degradation.

Also included within the scope of the resent invention are soluble forms of the TSG-14 protein, and

functional derivatives of the TSG-14 protein having similar bioactivity for all the uses described herein. Also intended are all active forms of TSG-14 derived from the TSG-14 transcript, and all muteins with TSG-14 activity.

By "functional derivative" is meant a "fragment," "variant," "analog," or "chemical derivative" of the TSG-14 protein. A functional derivative retains at least a portion of the function of the TSG-14 protein which permits its utility in accordance with the present invention.

A "fragment" of the TSG-14 protein is any subset of the molecule, that is, a shorter peptide.

A "variant" of the TSG-14 refers to a molecule substantially similar to either the entire peptide or a fragment thereof. Variant peptides may be conveniently prepared by direct chemical synthesis of the variant peptide, using methods well- known in the art.

Alternatively, amino acid sequence variants of the peptide can be prepared by mutations in the DNA which encodes the synthesized peptide. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity. Obviously, the mutations that will be made in the DNA encoding the variant peptide must not alter the reading frame and preferably will not create complementary regions that could produce secondary mRNA structure (see European Patent Publication No. EP 75,444).

At the genetic level, these variants ordinarily are prepared by site-directed mutagenesis (as exemplified by Adelman et al., DNA 2:183 (1983)) of nucleotides in the DNA encoding the peptide molecule, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. The variants typically exhibit the same qualitative biological activity as the nonvariant peptide.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector that includes within its sequence a DNA sequence that

encodes the relevant peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically, for example, by the method of Crea et al., Proc. Natl. Acad. Sci. (USA) 75:5765 (1978). This primer is then annealed with the single-stranded protein-sequence-containing vector, and subjected to DNA-polymerizing enzymes such as E. coli polymerase I Klenow fragment, to complete the synthesis of the mutation-bearing strand. Thus, a mutated sequence in the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells and clones are selected that include recombinant vectors bearing the mutated sequence arrangement.

The mutated protein region may be removed and placed in an appropriate vector for protein production, generally an expression vector of the type that may be employed for transformation of an appropriate host.

Alternatively, the DNA encoding a normal or variant TSG-14 protein can be altered by homologous recombination, a technique developed within the past few years for targeting genes to induce or correct mutations in transcriptionally active genes (Kucherlapati, Prog. in Nucl. Acid Res. and Mol. Biol. 36:301 (1989)). The technique of homologous recombination was developed as a method for introduction of specific mutations into specific regions of the mammalian genome (Thomas et al., Cell, 44:419-428, 1986; Thomas and Capecchi, Cell 51:503-512 (1987); Doetschman et al., Proc. Natl. Acad. Sci. USA 85:8583-8587 (1988)) or to correct specific mutations within defective genes (Doetschman et al., Nature 330:576-578 (1987)). The above references to homologous recombination are hereby incorporated by reference.

An example of a terminal insertion includes a fusion of a signal sequence, whether heterologous or homologous to the host cell, to the N-terminus of the peptide molecule to facilitate the secretion of mature peptide molecule from recombinant hosts.

Another group of variants are those in which at least one amino acid residue in the peptide molecule, and

preferably, only one, has been removed and a different residue inserted in its place. Such substitutions preferably are made in accordance with the following list when it is desired to modulate finely the characteristics of a peptide molecule.

Original Residue	Exemplary Substitutions	Original Residue	Exemplary Substitutions
Ala	gly; ser	Leu	ile; val
Arg	lys	Lys	arg; gln; glu
Asn	gln; his	Met	leu; tyr; ile
Asp	glu	Phe	met; leu; tyr
Cys	ser	Ser	thr
Gln	asn	Thr	ser
Glu	asp	Trp	tyr
Gly	ala; pro	Tyr	trp; phe
His	asn; gln	Val	ile; leu
Ile	leu; val		

Substantial changes in functional or immunological properties are made by selecting substitutions that are less conservative than those in the above list, that is, by selecting residues that differ more significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions that in general are expected to produce substantial changes are those in which (a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any other residue; (d) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) a residue having an electronegative charge, e.g., glutamyl or aspartyl; or (e) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Most deletions and insertions, and substitutions in particular, are not expected to produce radical changes in the characteristics of the peptide molecule. However, when it is

difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. For example, a TSG-14 variant typically is made by site-specific mutagenesis or homologous recombination of the TSG-14-encoding nucleic acid, expression of the variant nucleic acid in recombinant cell culture, and, optionally, purification from the cell culture, for example, by immunoaffinity adsorption on an antibody containing column.

An "analog" of the TSG-14 protein refers to a non-natural molecule substantially similar to either the entire molecule or a fragment thereof.

A "chemical derivative" of the TSG-14 protein contains additional chemical moieties not normally a part of the protein. Covalent modifications of the peptide are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

Cysteinyl residues most commonly are reacted with alpha-haloacetates (and corresponding amines), such as 2-chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, alpha-bromo- beta-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N- alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4- nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylprocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide alsc is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of

the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3- butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues per se has been studied extensively, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizol and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'-N-C-N-R') such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl) carbodiimide or 1- ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Derivatization with bifunctional agents is useful for cross-linking the peptide to a water-insoluble support matrix or to other macromolecular carriers. Commonly used cross-linking agents include, e.g., 1,1-bis(diazoacetyl)-2-

phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succin-imidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecule Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and, in some instances, amidation of the C-terminal carboxyl groups.

Such derivatized moieties may improve the solubility, absorption, biological half life, and the like. The moieties may alternatively eliminate or attenuate any undesirable side effect of the protein and the like. Moieties capable of mediating such effects are disclosed, for example, in Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980).

Standard reference works setting forth the general principles of recombinant DNA technology include Watson, J.D. et al., Molecular Biology of the Gene, Volumes I and II, The Benjamin/Cummings Publishing Company, Inc., publisher, Menlo Park, CA (1987); Darnell, J.E. et al., Molecular Cell Biology, Scientific American Books, Inc., publisher, New York, N.Y. (1986); Lewin, B.M., Genes II, John Wiley & Sons, publishers, New York, N.Y. (1985); Old, R.W., et al., Principles of Gene Manipulation: An Introduction to Genetic Engineering, 2d edition, University of California Press, publisher, Berkeley,

CA (1981); and Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). These references are hereby incorporated by reference.

By "cloning" is meant the use of in vitro recombination techniques to insert a particular gene or other DNA sequence into a vector molecule. In order to successfully clone a desired gene, it is necessary to employ methods for generating DNA fragments, for joining the fragments to vector molecules, for introducing the composite DNA molecule into a host cell in which it can replicate, and for selecting the clone having the target gene from amongst the recipient host cells.

By "cDNA" is meant complementary or copy DNA produced from an RNA template by the action of RNA-dependent DNA polymerase (reverse transcriptase). Thus a "cDNA clone" means a duplex DNA sequence complementary to an RNA molecule of interest, carried in a cloning vector.

By "cDNA library" is meant a collection of recombinant DNA molecules containing cDNA inserts which together comprise the entire expressible genome of an organism. Such a cDNA library may be prepared by methods known to those of skill, and described, for example, in Sambrook et al., supra. Generally, RNA is first isolated from the cells of an organism from whose genome it is desired to clone a particular gene. Preferred for the purposes of the present invention are mammalian, most preferably, human, cell lines.

Oligonucleotides representing a portion of the TSG-14 sequence are useful for screening for the presence of homologous genes and for the cloning of such genes. Techniques for synthesizing such oligonucleotides are disclosed by, for example, Wu, R., et al., Prog. Nucl. Acid. Res. Molec. Biol. 21:101-141 (1978).

Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid (Watson, J.D., In: Molecular Biology of the Gene, 4th Ed., Benjamin/Cummings Publishing Co., Inc., Menlo Park, CA

(1987)). Using the genetic code, one or more different oligonucleotides can be identified, each of which would be capable of encoding the amino acid. The probability that a particular oligonucleotide will, in fact, constitute the actual XXX-encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic cells. Such "codon usage rules" are disclosed by Lathe, R., et al., J. Molec. Biol. 183:1-12 (1985). Using the "codon usage rules" of Lathe, a single oligonucleotide, or a set of oligonucleotides, that contains a theoretical "most probable" nucleotide sequence capable of encoding the TSG-14 sequences is identified.

Although occasionally an amino acid sequence may be encoded by only a single oligonucleotide, frequently the amino acid sequence may be encoded by any of a set of similar oligonucleotides. Importantly, whereas all of the members of this set contain oligonucleotides which are capable of encoding the TSG-14 peptide fragment and, thus, potentially contain the same oligonucleotide sequence as the gene which encodes the peptide fragment, only one member of the set contains the nucleotide sequence that is identical to the nucleotide sequence of the gene. Because this member is present within the set, and is capable of hybridizing to DNA even in the presence of the other members of the set, it is possible to employ the unfractionated set of oligonucleotides in the same manner in which one would employ a single oligonucleotide to clone the gene that encodes the protein.

The oligonucleotide, or set of oligonucleotides, containing the theoretical "most probable" sequence capable of encoding the TSG-14 fragment is used to identify the sequence of a complementary oligonucleotide or set of oligonucleotides which is capable of hybridizing to the "most probable" sequence, or set of sequences. An oligonucleotide containing such a complementary sequence can be employed as a probe to identify and isolate the TSG-14 gene (Sambrook et al., supra).

A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the TSG-14 gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified (using the above-described procedure), synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA preparation derived from cells which are capable of expressing the TSG-14 gene, such as TNF-treated FS-4 cells.

Single stranded oligonucleotide molecules complementary to the "most probable" TSG-14 protein coding sequences can be synthesized using procedures which are well known to those of ordinary skill in the art (Belagaje, R., et al., J. Biol. Chem. 254:5765-5780 (1979); Maniatis, T., et al., In: Molecular Mechanisms in the Control of Gene Expression, Nierlich, D.P., et al., Eds., Acad. Press, NY (1976); Wu, R., et al., Prog. Nucl. Acid Res. Molec. Biol. 21:101-141 (1978); Khorana, R.G., Science 203:614-625 (1979)). Additionally, DNA synthesis may be achieved through the use of automated synthesizers. Techniques of nucleic acid hybridization are disclosed by Sambrook et al. (supra), and by Haymes, B.D., et al. (In: Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, DC (1985)), which references are herein incorporated by reference. Techniques such as, or similar to, those described above have successfully enabled the cloning of genes for human aldehyde dehydrogenases (Hsu, L.C., et al., Proc. Natl. Acad. Sci. USA 82:3771-3775 (1985)), fibronectin (Suzuki, S., et al., Eur. Mol. Biol. Organ. J. 4:2519-2524 (1985)), the human estrogen receptor gene (Walter, P., et al., Proc. Natl. Acad. Sci. USA 82:7889-7893 (1985)), tissue-type plasminogen activator (Pennica, D., et al., Nature 301:214-221 (1983)) and human term placental alkaline phosphatase complementary DNA (Kam, W., et al., Proc. Natl. Acad. Sci. USA 82:8715-8719 (1985)).

In an alternative way of cloning the TSG-14 gene, a library of expression vectors is prepared by cloning DNA or, more preferably, cDNA (from a cell capable of expressing TSG-14, such as a TNF-treated FS-4 cell) into an expression

vector. The library is then screened for members capable of expressing a protein which binds to anti-TSG-14 antibody, and which has a nucleotide sequence that is capable of encoding polypeptides that have the same amino acid sequence as TSG-14 proteins or peptides, or fragments thereof. In this embodiment, DNA, or more preferably cDNA, is extracted and purified from a cell which is capable of expressing TSG-14 protein. The purified cDNA is fragmentized (by shearing, endonuclease digestion, etc.) to produce a pool of DNA or cDNA fragments. DNA or cDNA fragments from this pool are then cloned into an expression vector in order to produce a genomic library of expression vectors whose members each contain a unique cloned DNA or cDNA fragment.

By "vector" is meant a DNA molecule, derived from a plasmid or bacteriophage, into which fragments of DNA may be inserted or cloned. A vector will contain one or more unique restriction sites, and may be capable of autonomous replication in a defined host or vehicle organism such that the cloned sequence is reproducible.

An "expression vector" is a vector which (due to the presence of appropriate transcriptional and/or translational control sequences) is capable of expressing a DNA (or cDNA) molecule which has been cloned into the vector and of thereby producing a polypeptide or protein. Expression of the cloned sequences occurs when the expression vector is introduced into an appropriate host cell. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences. Similarly, if a eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequences. Importantly, since eukaryotic DNA may contain intervening sequences, and since such sequences cannot be correctly processed in prokaryotic cells, it is preferable to employ cDNA from a cell which is capable of expressing TSG-14 in order to produce a prokaryotic genomic expression vector library. Procedures for preparing cDNA and for producing a genomic library are disclosed by Sambrook et al. (supra).

By "functional derivative" of a polynucleotide (DNA or RNA) molecule is meant a polynucleotide molecule encoding a "fragment" or "variant" of the TSG-14 protein. It can be a chemical derivative which retains its functions such as the ability to hybridize with a complementary polynucleotide molecule. Such a polynucleotide, or oligonucleotide, chemical derivative is useful as a molecular probe to detect TSG-14 sequences through nucleic acid hybridization assays.

A DNA sequence encoding the TSG-14 protein of the present invention, or its functional derivatives, may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed by Sambrook, J. et al., supra, and are well known in the art.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal the initiation of protein synthesis. Such regions will normally include those 5'- non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the gene sequence coding for the protein may be obtained by the above-

described methods. This region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence coding for the protein, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted.

Two sequences of a nucleic acid molecule are said to be "operably linked" when they are linked to each other in a manner which either permits both sequences to be transcribed onto the same RNA transcript, or permits an RNA transcript, begun in one sequence to be extended into the second sequence. Thus, two sequences, such as a promoter sequence and any other "second" sequence of DNA or RNA are operably linked if transcription commencing in the promoter sequence will produce an RNA transcript of the operably linked second sequence. In order to be "operably linked" it is not necessary that two sequences be immediately adjacent to one another.

A promoter is a double-stranded DNA or RNA molecule which is capable of binding RNA polymerase and promoting the transcription of an "operably linked" nucleic acid sequence. As used herein, a "promoter sequence" is the sequence of the promoter which is found on that strand of the DNA or RNA which is transcribed by the RNA polymerase. A "promoter sequence complement" is a nucleic acid molecule whose sequence is the complement of a "promoter sequence." Hence, upon extension of a primer DNA or RNA adjacent to a single-stranded "promoter sequence complement" or, of a "promoter sequence," a double-stranded molecule is created which will contain a functional promoter, if that extension proceeds towards the "promoter sequence" or the "promoter sequence complement." This functional promoter will direct the transcription of a nucleic acid molecule which is operably linked to that strand of the double-stranded molecule which contains the "promoter sequence" (and not that strand of the molecule which contains the "promoter sequence complement").

Certain RNA polymerases exhibit a high specificity for such promoters. The RNA polymerases of the bacteriophages T7, T3, and SP-6 are especially well characterized, and exhibit high promoter specificity. The promoter sequences which are specific for each of these RNA polymerases also direct the polymerase to utilize (i.e. transcribe) only one strand of the two strands of a duplex DNA template. The selection of which strand is transcribed is determined by the orientation of the promoter sequence. This selection determines the direction of transcription since RNA is only polymerized enzymatically by the addition of a nucleotide 5' phosphate to a 3' hydroxyl terminus. The promoter sequences of the present invention may be either prokaryotic, eukaryotic or viral. Suitable promoters are repressible, or, more preferably, constitutive. Strong promoters are preferred.

The present invention encompasses the expression of the TSG-14 protein (or a functional derivative thereof) in either prokaryotic or eukaryotic cells, although eukaryotic expression is preferred.

Preferred prokaryotic hosts include bacteria such as E. coli, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia, etc. The most preferred prokaryotic host is E. coli. Other enterobacteria such as Salmonella typhimurium or Serratia marcescens, and various Pseudomonas species may also be utilized. Under such conditions, the protein may not be glycosylated. The procaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

The TSG-14 protein can be expressed in a prokaryotic cell (such as, for example, E. coli, B. subtilis, Pseudomonas, Streptomyces, etc.), either by itself, or as part of a fusion protein. For expression as a fusion protein, it must be linked in the appropriate reading fram with a prokaryotic protein. Preferred fusion protein "partners" are the trpE protein of E. coli or a bacteriophage protein, such as that of the MS2 phage (see Examples, below). To express the TSG-14 protein (or a functional derivative thereof) in a prokaryotic host, it is necessary to operably link the TSG-14 encoding

sequence to a functional prokaryotic promoter. Examples of constitutive promoters include the int promoter of bacteriophage lambda, the bla promoter of the ß-lactamase gene of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene of pBR325, etc. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage lambda (P_L and P_R), the trp, recA, lacZ, lacI, and gal promoters of E. coli, the α-amylase (Ulmanen, I., et al., J. Bacteriol. 162:176-182 (1985)) and the s-28-specific promoters of B. subtilis (Gilman, M.Z., et al., Gene 32:11-20 (1984)), the promoters of the bacteriophages of Bacillus (Gryczan, T.J., In: The Molecular Biology of the Bacilli, Academic Press, Inc., NY (1982)), and Streptomyces promoters (Ward, J.M., et al., Mol. Gen. Genet. 203:468-478 (1986)). Prokaryotic promoters are reviewed by Glick, B.R., (J. Ind. Microbiol. 1:277-282 (1987)); Cenatiempo, Y. (Biochimie 68:505-516 (1986)); and Gottesman, S. (Ann. Rev. Genet. 18:415-442 (1984)). For the present invention, a most preferred promoter is the PL promoter of lambda; alternatively, the protein can be expressed under control of a temperature-sensitive repressor of the lambda PL promoter.

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene- encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold, L., et al. (Ann. Rev. Microbiol. 35:365-404 (1981)).

Preferred hosts are eukaryotic hosts including yeast, insects, fungi, and mammalian cells either in vivo, or in tissue culture. Mammalian cells provide post-translational modifications to protein molecules including correct folding or glycosylation at correct sites. Mammalian cells which may be useful as hosts include cells of fibroblast origin such as VERO or CHO, or cells of lymphoid origin, such as the hybridoma SP2/O-Ag14 or the murine myeloma P3-X63Ag8, and their derivatives. A most preferred host is one that does not express the TSG-14 gene upon treatment with TNF, such as GM-637, a SV40-transformed human fibroblast cell line.

For a mammalian cell host, many possible vector systems are available for the expression of TSG-14. A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, Simian virus, or the like, where the regulatory signals are associated with a particular gene which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, etc., may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the genes can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical regulation, e.g., metabolite.

A yeast cell host provides substantial advantages in that it can also carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides).

Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeast are grown in mediums rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcriptional control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase gene can be utilized.

Production of TSG-14 or functional derivatives thereof in insects can be achieved, for example, by infecting the insect host with a baculovirus engineered to express TSG-14 by methods known to those of skill. Thus, in one

embodiment, sequences encoding TSG-14 may be operably linked to the regulatory regions of the viral polyhedrin protein (Jasny, Science 238: 1653 (1987)). Infected with the recombinant baculovirus, cultured insect cells, or the live insects themselves, can produce the TSG-14 protein in amounts as great as 20 to 50% of total protein production. When live insects are to be used, caterpillars are presently preferred hosts for large scale TSG-14 production according to the invention.

As discussed above, expression of the TSG-14 protein in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include the the SV40 early promoter (Benoist, C., et al., Nature (London) 290:304-310 (1981)); the RSV promoter associated with an MMTV LTR region; promoter of the mouse metallothionein I gene (Hamer, D., et al., J. Mol. Appl. Gen. 1:273-288 (1982)); the TK promoter of Herpes virus (McKnight, S., Cell 31:355-365 (1982)); the yeast gal4 gene promoter (Johnston, S.A., et al., Proc. Natl. Acad. Sci. (USA) 79:6971-6975 (1982); Silver, P.A., et al., Proc. Natl. Acad. Sci. (USA) 81:5951-5955 (1984)).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes the TSG-14 protein (or a functional derivative thereof) does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as TSG-14 encoding DNA sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the TSG-14 encoding sequence).

The TSG-14 encoding sequence and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more

preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the TSG-14 protein may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced sequence into the host chromosome.

In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototropy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, H., Mol. Cell. Biol. 3:28 (1983).

In a preferred embodiment, the introduced sequence will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in E. coli (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX. Such plasmids are, for example,

disclosed by Sambrook et al. (supra). Bacillus plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan, T. (In: The Molecular Biology of the Bacilli, Academic Press, NY (1982), pp. 307-329). Suitable Streptomyces plasmids include pIJ101 (Kendall, K.J., et al., J. Bacteriol. 169:4177-4183 (1987)), and streptomyces bacteriophages such as φC31 (Chater, K.F., et al., In: Sixth International Symposium on Actinomycetales Biology, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). Pseudomonas plasmids are reviewed by John, J.F., et al. (Rev. Infect. Dis. 8:693-704 (1986)), and Izaki, K. (Jpn. J. Bacteriol. 33:729-742 (1978)).

Preferred eukaryotic plasmids include BPV, vaccinia, SV40, 2-micron circle, etc., or their derivatives. Such plasmids are well known in the art (Botstein, D., et al., Miami Wntr. Symp. 19:265-274 (1982); Broach, J.R., In: The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, J.R., Cell 28:203-204 (1982); Bollon, D.P., et al., J. Clin. Hematol. Oncol. 10:39-48 (1980); Maniatis, T., In: Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Expression, Academic Press, NY, pp. 563-608 (1980)).

Once the vector or DNA sequence containing the construct(s) has been prepared for expression, the vector or DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment (Johnston et al., Science 240:1538 (1988)), etc.

After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of the TSG-14 protein, or in the production of a fragment of this protein.

This can take place in the transformed cells as such, or following the induction of these cells to differentiate.

The expressed protein or fusion protein may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. For example, the cells may be collected by centrifugation, or with suitable buffers, lysed, and the protein isolated by column chromatography, for example, on DEAE-cellulose, phosphocellulose, polyribocytidylic acid-agarose, hydroxyapatite or by electrophoresis or immunoprecipitation. Alternatively, the TSG-14 or functional derivative thereof may be isolated by the use of anti- TSG-14 antibodies. Such antibodies may be obtained by well-known methods, some of which are mentioned below.

Genetic constructs encoding TSG-14 functional derivatives thereof such as those described above, can be used in gene therapy. An abnormal TSG-14 molecule which results in enhanced susceptibility to disease, may be replaced by infusion of cells of the desired lineage (such as fibroblasts, for example) transfected with DNA encoding normal or modified TSG-14 protein, under conditions where the infused cells will preferentially replace the endogenous cell population.

The present invention is also directed to a transgenic non-human eukaryotic animal (preferably a rodent, such as a mouse) the germ cells and somatic cells of which contain genomic DNA according to the present invention which encodes the TSG-14 protein or a functional derivative thereof. The TSG-14 DNA is introduced into the animal to be made transgenic, or an ancestor of the animal, at an embryonic stage, preferably the one-cell, or fertilized oocyte, stage, and generally not later than about the 8-cell stage. The term "transgene," as used herein, means a gene which is incorporated into the genome of the animal and is expressed in the animal, resulting in the presence of protein in the transgenic animal.

There are several means by which such a gene can be introduced into the genome of the animal embryo so as to be

chromosomally incorporated and expressed. One method is to transfect the embryo with the gene as it occurs naturally, and select transgenic animals in which the gene has integrated into the chromosome at a locus which results in expression. Other methods for ensuring expression involve modifying the gene or its control sequences prior to introduction into the embryo. One such method is to transfect the embryo with a vector (see above) containing an already modified gene. Other methods are to use a gene the transcription of which is under the control of a inducible or constitutively acting promoter, whether synthetic or of eukaryotic or viral origin, or to use a gene activated by one or more base pair substitutions, deletions, or additions (see above).

Introduction of the desired gene sequence at the fertilized oocyte stage ensures that the transgene is present in all of the germ cells and somatic cells of the transgenic animal and has the potential to be expressed in all such cells. The presence of the transgene in the germ cells of the transgenic "founder" animal in turn means that all its progeny will carry the transgene in all of their germ cells and somatic cells. Introduction of the transgene at a later embryonic stage in a founder animal may result in limited presence of the transgene in some somatic cell lineages of the founder; however, all the progeny of this founder animal that inherit the transgene conventionally, from the founder's germ cells, will carry the transgene in all of their germ cells and somatic cells.

Chimeric non-human mammals in which fewer than all of the somatic and germ cells contain the TSG-14 DNA of the present invention, such as animals produced when fewer than all of the cells of the morula are transfected in the process of producing the transgenic mammal, are also intended to be within the scope of the present invention.

The techniques described in Leder, U.S. Patent 4,736,866 (hereby incorporated by reference) for producing transgenic non-human mammals may be used for the production of the transgenic non-human mammal of the present invention. The various techniques described in Palmiter, R. et al., Ann. Rev.

Genet. 20:465-99 (1986), the entire contents of which are hereby incorporated by reference, may also be used.

The animals carrying the TSG-14 gene can be used to test compounds or other treatment modalities which may prevent, suppress or cure chronic inflammatory conditions mediated by TNF action on connective tissue cells. These tests can be extremely sensitive because of the ability to adjust the dose of an agent under test given to the transgenic animals of this invention. Such diseases include, but are not limited to rheumamtoid arthritis, granulomatous diseases, and the like. Transgenic animals according to the present invention can also be used as a source of cells for cell culture.

This invention is also directed to an antibody specific for an epitope of TSG-14 protein. In additional embodiments, the antibodies of the present invention are used in methods to detect the presence of, or measure the quantity or concentration of, TSG-14 protein in a cell, or in a cell or tissue extract, or a biological fluid. The antibodies may also be used in methods for measuring induction of expression of TSG-14 in a cell or in methods for identifying a compound capable of inducing the expression of TSG-14 in a cell. The antibodies may also be used to disrupt the action of TSG-14, thereby preventing or treating diseases associated with overproduction, or inappropriate production or action of TSG-14, such as inflammatory disorders including rheumatoid arthritis, infections and sepsis, as well as conditions associated with TNF-stimulated leukocyte adhesion.

The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, and anti-idiotypic (anti-Id) antibodies.

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface

groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one, or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

In order to predict antigenic epitopes present in the protein, the amino acid sequence is inspected visually or analyzed by computer, for example, using the program of PEPTIDESTRUCTURE (Jameson et al., CABIOS 4: 181-186 (1988)). This program allows determination of hydropathicity values which are then used to determine which peptide sequences within the overall protein sequence are likely to be most immunogenic based on their potential secondary structure. Such peptides may be synthesized chemically, or alternatively, and preferably, by recombinant DNA methods.

One of the pitfalls of generating antibodies to synthetic peptides is the possibility that an antibody so raised may fail to react with the native protein. For this reason, alternative approaches may be used. The TSG-14 protein may be prepared as a bacterially expressed fusion protein by using an expression plasmid, such as the pATH2-TSG-14 expression vector described in Figure 7 and Example VII, below. Alternatively, the TSG-14 may be expressed as a fusion protein using an expression plasmid such as, for example, pDB169 (Lim, D. et al., supra)). The ArgR repressor protein is highly expressed in E. coli strain JM101 under the control of a strong tac promoter of this latter vector. The C-terminal portion of the ArgR coding region can be replaced by an N-terminally deleted TSG-14 coding sequence in the correct reading frame. This construct can then be transfected, for example, into JM101 cells, and the expression of the fusion

protein is monitored by SDS-PAGE. Expression of the fusion protein is induced with IPTG, specific for the tac promoter, in a large culture system and purified by affinity chromatography using antibodies specific for the ArgR repressor.

The purified fusion protein is employed for the immunization of rabbits. Alternatively, such a fusion protein, or a functional derivative of the protein such as a synthetic peptide may be used to immunize a rodent for generation of a monoclonal antibody.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen.

Monoclonal antibodies are a substantially homogeneous population of antibodies to specific antigens. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, Nature 256:495-497 (1975) and U.S. Patent No. 4,376,110. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and any subclass thereof. The hybridoma producing the mAbs of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo production makes this the presently preferred method of production. Briefly, cells from the individual hybridomas are injected intraperitoneally into pristane-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies and methods for their production are known in the art (Cabilly et al, Proc. Natl. Acad. Sci. USA 81:3273-3277 (1984); Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984); Boulianne et al., Nature 312:643-646 (1984); Neuberger et al.,

Nature 314:268-270 (1985); Taniguchi et al., European Patent Application 171496 (published February 19, 1985); Kudo et al., European Patent Application 184187 (published June 11, 1986); Robinson et al., International Patent Publication #PCT/US86/02269 (published 7 May 1987); Sun et al., Proc. Natl. Acad. Sci. USA 84:214-218 (1987); Better et al., Science 240:1041- 1043 (1988)). These references are hereby incorporated by reference.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the mAb with the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody).

The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may bear structural similarity to the original mAb which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against the TSG-14 protein of the present invention may be used to induce anti-Id antibodies in suitable animals, such as Balb/c mice. Spleen cells from such immunized mice are used to produce anti-Id hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional Balb/c mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original mAb specific for an TSG-14 protein epitope.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for

example, Fab and F(ab')2, which are capable of binding antigen. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)). It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of TSG-14 protein according to the methods disclosed herein for intact antibody molecules. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments).

The antibodies, or fragments of antibodies, of the present invention may be used to quantitatively or qualitatively detect the presence of cells which express the TSG-14 protein on their surface or intracellularly. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with light microscopic, flow cytometric, or fluorimetric detection. The antibodies of the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for in situ detection of TSG-14 protein. In situ detection may be accomplished by removing a histological (cell or tissue) specimen from a subject and providing the a labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying on the biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the TSG-14 protein but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

Additionally, the antibody of the present invention can be used to detect the presence of soluble TSG-14 molecules in a biological sample. Used in this manner, the antibody can

serve as a means to monitor the presence and quantity of TSG-14 proteins in a subject having a condition associated with TNF induction of TSG-14, such as an inflammatory condition, an infection or sepsis, and the like.

Such immunoassays for TSG-14 protein typically comprise incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested cells such as lymphocytes or leucocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying TSG-14 protein, and detecting the antibody by any of a number of techniques well-known in the art.

The biological sample may be treated with a solid phase support or carrier (which terms are used interchangeably herein) such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled TSG-14-specific antibody. The solid phase support may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on said solid support may then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding antigen or antibodies. Well-known supports, or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Those skilled in the art will know many other suitable carriers for binding

antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of anti-TSG-14 antibody may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

One of the ways in which the TSG-14-specific antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect TSG-14 protein through the use of a radioimmunoassay (RIA) (Chard, T., "An Introduction to Radioimmune Assay and Related Techniques" (In: Work, T.S., et al., Laboratory Techniques in Biochemistry in Molecular Biology, North Holland Publishing Company, New York (1978), incorporated by reference herein).

The radioactive isotope can be detected by such means as the use of a gamma counter or a liquid scintillation counter or by autoradiography. It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o- phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

The antibody molecules of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support and a quantity of detectably labeled soluble antibody is added to

permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

Typical, and preferred, immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to "extract" the antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support is washed to remove the residue of the fluid sample, including unreacted antigen, if any, and then contacted with the solution containing an unknown quantity of labeled antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the antigen bound to the solid support through the unlabeled antibody, the solid support is washed a second time to remove the unreacted labeled antibody.

In another type of "sandwich" assay, which may also be useful with the antigens of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the antibody bound to the solid support and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays.

According to the present invention, it is possible to diagnose circulating antibodies in a subject which are specific for the TSG-14 protein. This is accomplished by means of an immunoassay, as described above, using the protein of the invention or a functional derivative thereof.

In cancer patients, circulating endotoxin levels are high (Harris, R.I. et al., J. Clin. Path. 37:467-470 (1984)), particularly in patients with tumor types known to be associated with an increased incidence of cachexia (Humberstone, D.A. et al., Cancer 62:1619-1624 (1988)). The presence of high endotoxin levels is probably not a direct result of the tumor per se, but rather reflects the general debility of the patients. Increased translocation from the gut of endogenous bacteria and endotoxins in critical illness is dependent on the presence of malnutrition and that impaired cell-mediated immunity may be an aggravating factor (Wilmore, D.W. et al., Surgery 104:917-923 (1988)). As cachectic cancer patients are malnourished and often exhibit suppression of cell-mediated immunity, translocation of endogenous organisms may account for higher levels of endotoxins. Cancer patients' peripheral blood mononuclear cells often show enhanced "spontaneous" TNF release in vitro (Aderka, D. et al., Lancet i:1190-1192 (1985)). TNF production in response to macrophage-activating agents is reduced in patients with advanced metastatic disease but not in cancer patients with only localized disease. These observations supported the notion that TNF production is ongoing in cancer patients, either due to sestained stimulation of monocytes/macrophages by tumor cells or to direct TNF production by tumor cells. TNF was detected in the serum of 50% of 226 cancer patients with active disease, compared to 3% of healthy sera and 18% of sera from disease-free cancer patients (Balkwill, F. et al., Lancet ii: 1229-1232 (1987)).

TNF levels are also elevated in a variety of bacterial and viral illnesses, including AIDS (Lahdevirta, J. et al., Amer. J. Med. 85:289-291 (1988)) and meningococcal meningitis and septicemia (Waage, A. et al., (Lancet i:355-357 (1987)). In a rat burn/infection model, levels of hepatic TNF

mRNA increased 100% in rats subjected to burn + infection compared to controls or rats subjected to burns but no infection (Marano, M.A. et al., Arch. Surg. 123:1383-1388 (1988)). The animals subjected to burn and infection also showed a greater metabolic response (cachexia). Michie, H.R. et al., Br. J. Surg. 76:670-671 (1989), reviewed evidence that TNF is the principal mediator associated with the changes of severe sepsis.

Therefore, the methods of the present invention which are capable of measuring the response of a subject to a cytokine such as TNF or IL-1, or to bacterial endotoxin are useful in predicting the susceptibility of that individual to the debilitating effects of cancer or infectious disease. Similarly, the compositions of the present invention are useful in the prevention or treatment of such diseases, due to their ability to disrupt events set into motion by the action of TNF.

As used herein, the term "prevention" of a condition, such as an inflammatory response, in a subject involves administration of the TSG-14 peptide derivative, or antibody (see above) prior to the clinical onset of the disease. "Treatment" involves administration of the protective composition after the clinical onset of the disease. For example, successful administration of a TSG-14 peptide derivative or anti-TSG-14 antibody according to the invention after development of an inflammatory condition, a malignant tumor or an infection comprises "treatment" of the disease.

The TSG-14 protein, peptides or antibody of the present invention may be administered by any means that achieve their intended purpose, for example, to treat rheumatoid arthritis or other inflammatory conditions, malignant tumors, infections and the like.

For example, administration may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the topical route or

the oral route. Parenteral administration can be by bolus injection or by gradual perfusion over time.

A typical regimen for preventing, suppressing, or treating a condition such chronic inflammation (as in rheumatoid arthritis) or a malignant tumor, comprises administration of an effective amount of the TSG-14 functional derivative, or an antibody thereto, administered over a period of one or several days, up to and including between one week and about six months. It is understood that the dosage administered will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to be limiting and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art.

The total dose required for each treatment may be administered by multiple doses or in a single dose. The protein, functional derivative thereof or antibody may be administered alone or in conjunction with other therapeutics directed to the viral infection, or directed to other symptoms of the viral disease.

Effective amounts of the TSG-14 protein, functional derivative thereof, or antibody thereto, are from about 0.01 μg to about 100 mg/kg body weight, and preferably from about 10 μg to about 50 mg/kg body weight.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets and capsules can also be prepared according to routine methods.

Pharmaceutical compositions comprising the proteins, peptides or antibodies of the inventioninclude all compositions wherein the protein, peptide or antibody is contained in an amount effective to achieve its intended purpose. In addition, the pharmaceutical compositions may

contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Pharmaceutical compositions include suitable solutions for administration by injection or orally, and contain from about 0.01 to 99 percent, preferably from about 20 to 75 percent of active component (i.e., the TSG-14 protein or antibody) together with the excipient. Pharmaceutical compositions for oral administration include tablets and capsules. Compositions which can be administered rectally include suppositories.

The preferred animal subject of the present invention is a mammal. By the term "mammal" is meant an individual belonging to the class Mammalia. The invention is particularly useful in the treatment of human subjects, although it is intended for veterinary uses as well.

The present invention provides methods for evaluating the presence and the level of normal or mutant TSG-14 protein or mRNA in a subject. For example, over-expression of TSG-14 in response to stimulation with TNF, IL-1 or an exogenous stimulus such as a bacterial infection, may serve as an important predictor of the inflammatory or septic response. By providing a means to measure the quantity of TSG-14 mRNA in a hybridization assay or TSG-14 protein, as in an immunoassay, the present invention provides a means for detecting susceptibility in a subject to development of an inflammatory condition, such as rheumatoid arthritis, to infectious and septic conditions, and the like.

Oligonucleotide probes encoding various portions of the TSG-14 DNA sequence are used to test cells from a subject for the presence TSG-14 DNA or mRNA. A preferred probe would be one directed to the nucleic acid sequence encoding at least 12 and preferably at least 15 nucleotides of the TSG-14 sequence. Qualitative or quantitative assays can be performed using such probes. For example, Northern analysis (see

Examples below) is used to measure expression of an TSG-14 mRNA in a cell or tissue preparation.

Such methods can be used even with very small amounts of DNA obtained from an individual, following use of selective amplification techniques. Recombinant DNA methodologies capable of amplifying purified nucleic acid fragments have long been recognized. Typically, such methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by Cohen et al. (U.S. Patent 4,237,224), Sambrook et al. (supra), etc.

Recently, an in vitro enzymatic method has been described which is capable of increasing the concentration of such desired nucleic acid molecules. This method has been referred to as the "polymerase chain reaction" or "PCR" (Mullis, K. et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); Erlich H. et al., EP 50,424; EP 84,796, EP 258,017, EP 237,362; Mullis, K., EP 201,184; Mullis K. et al., US 4,683,202; Erlich, H., US 4,582,788; and Saiki, R. et al., US 4,683,194).

The polymerase chain reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotide probes to serve as primers for the template-dependent, polymerase mediated replication of a desired nucleic acid molecule.

The precise nature of the two oligonucleotide probes of the PCR method is critical to the success of the method. As is well known, a molecule of DNA or RNA possesses directionality, which is conferred through the 5'-3' linkage of the phosphate groups of the molecule. Sequences of DNA or RNA are linked together through the formation of a phosphodiester bond between the terminal 5' phosphate group of

one sequence and the terminal 3' hydroxyl group of a second sequence. Polymerase dependent amplification of a nucleic acid molecule proceeds by the addition of a 5' nucleotide triphosphate to the 3' hydroxyl end of a nucleic acid molecule. Thus, the action of a polymerase extends the 3' end of a nucleic acid molecule. These inherent properties are exploited in the selection of the oligonucleotide probes of the PCR. The oligonucleotide sequences of the probes of the PCR method are selected such that they contain sequences identical to, or complementary to, sequences which flank the particular nucleic acid sequence whose amplification is desired.

More specifically, the oligonucleotide sequences of the "first" probe is selected such that it is capable of hybridizing to an oligonucleotide sequence located 3' to the desired sequence, whereas the oligonucleotide sequence of the "second" probe is selected such that it contains an oligonucleotide sequence identical to one present 5' to the desired region. Both probes possess 3' hydroxy groups, and therefore can serve as primers for nucleic acid synthesis. In the PCR, the reaction conditions are cycled between those conducive to hybridization and nucleic acid polymerization, and those which result in the denaturation of duplex molecules. In the first step of the reaction, the nucleic acids of the sample are transiently heated, and then cooled, in order to denature any double-stranded molecules which may be present. The "first" and "second" probes are then added to the sample at a concentration which greatly exceeds that of the desired nucleic acid molecule. When the sample is incubated under conditions conducive to hybridization and polymerization, the "first" probe will hybridize to the nucleic acid molecule of the sample at a position 3' to the sequence to be amplified. If the nucleic acid molecule of the sample was initially double-stranded, the "second" probe will hybridize to the complementary strand of the nucleic acid molecule at a position 3' to the sequence which is the complement of the sequence whose amplification is desired. Upon addition of a polymerase, the 3' ends of the

"first" and (if the nucleic acid molecule was double-stranded) "second" probes will be extended. The extension of the "first" probe will result in the synthesis of an oligonucleotide having the exact sequence of the desired nucleic acid. Extension of the "second" probe will result in the synthesis of an oligonucleotide having the exact sequence of the complement of the desired nucleic acid.

The PCR reaction is capable of exponential amplification of specific nucleic acid sequences because the extension product of the "first" probe, of necessity, contains a sequence which is complementary to a sequence of the "second" probe, and thus can serve as a template for the production of an extension product of the "second" probe. Similarly, the extension product of the "second" probe, of necessity, contains a sequence which is complementary to a sequence of the "first" probe, and thus can serve as a template for the production of an extension product of the "first" probe. Thus, by permitting cycles of polymerization, and denaturation, a geometric increase in the concentration of the desired nucleic acid molecule can be achieved. Reviews of the PCR are provided by Mullis, K.B. (Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986)); Saiki, R.K., et al. (Bio/Technology 3:1008-1012 (1985)); and Mullis, K.B., et al. (Meth. Enzymol. 155:335-350 (1987)).

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE I

Preparation of cDNA Library from TNF-Treated FS-4 Cells and Isolation of TNF-Inducible cDNA

Materials

E. coli-derived recombinant human TNF (specific activity, 3 x 10^7 U/mg) was supplied by M. Tsujimoto of the Suntory Institute for Biomedical Research, Osaka, Japan. E. coli-derived recombinant human IL-1α (specific activity, 1 x 10^9 U/mg) was received from Alvin Stern and Peter Lomedico, Hoffmann-LaRoche, Inc., Nutley, NJ. E. coli-derived human gamma interferon (IFN-gamma) (specific activity, 2.1 x 10^7 U/mg) was provided by Biogen, Cambridge, MA. E. coli-derived human IFN-ß (Betaseron, specific activity, 2 x 10^8 U/mg) was obtained from Triton Biosciences, Alameda, CA. Epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and transforming growth factor-ß (TGF-ß) were purchased from Collaborative Research, INc., Bedford, MA. Poly(I)-poly(C) was from P-L Biochemicals, Inc., Milwaukee, WI. N^6-2'-O-dibutyl adenosine cyclic 3',5'-monophosphate, cycloheximide, forskolin, 12-O-tetradecanoylphorbol 13-acetate (TPA), the calcium ionophore A23187, and isobutylmethylxanthine were purchased from Sigma Chemical Co., St. Louis, MO. The pHe7 plasmid, used as a source of internal reference cDNA (Kaczmarek, L. et al., J. Cell Biol. 104:183-187 (1987), was supplied by P.B. Sehgal, Rockefeller University, New York, NY.

Cell Culture

The human diploid FS-4 foreskin fibroblast cell line (Vilcek, J. et al., Proc. Natl. Acad. Sci. USA 70:3909-3913 (1973)) was used at passage level 15 in all experiments. FS-4 cells were grown in Eagle minimal essential medium (E-MEM) supplemented with 6 mM HEPES, 3 mM Tricine, 50 µg/ml gentamicin, and 5% heat inactivated (56°C, 30 min) fetal bovine serum (FBS; GIBCO Laboratories, Grand Island, NY). For experiments, 4 x 10^6 cells were seeded in 175 cm^2 Falcon flasks, incubated at 37°C, and allowed to grow to confluence over 6 days. The confluent monolayers were washed once with phosphate buffered saline and replenished with E-MEM

containing 0.25% FBS. The cultures were incubated in this medium for another 72 h at 37°C to let the cells become quiescent and then treated with the appropriate agents, as specified herein.

Preparation of cDNA and Construction of cDNA Library

Total cytoplasmic RNA was isolated from quiescent FS-4 cells treated for 3 h with TNF (10 ng/ml) as described previously (Lin, J.-X. et al., J. Biol. Chem. 262:11908-11911 (1987)). Poly(A)$^+$ RNA was selected by one cycle of binding to oligo(dT)-cellulose (type 7; P-L Biochemicals). Double stranded cDNA was made from 10 μg of poly(A)$^+$ by using the cDNA synthesis system of Bethesda Research Laboratories, Gaithersburg, MD. The double stranded cDNA was methylated with EcoRI methylase and made blunt-ended with T4 DNA polymerase. EcoRI linkers were ligated onto the cDNA, which was then restricted with EcoRI. The resulting cDNA greater than 600 base pairs in size was fractionated and separated from the linker fragments by Sepharose CL4B column chromatography and ligated into the EcoRI site of lambda gt10. The library was packed in vitro with Gigapack packaging extract (Stratagene).

Differential Screening of the cDNA Library

The lambda gt10 cDNA library was plated on E. coli LE392 at a density of 1000 PFU/dish (150 mm diameter). Nitrocellulose filters were used to prepare duplicate plaque lifts of each plats. Prehybridization and hybridization of filters with ^{32}P-labeled single-stranded cDNA probe were performed as described (Leonard, D.G.B. et al., Molec. Cell. Biol. 7:3156-3167 (1987)). Probes were synthesized by using the Bethesda Research Laboratories cDNA synthesis system with 10 μg poly(A)$^+$ RNA. The first strand synthesis reaction was adjusted to contain 0.5 mM each of dATP, dGTP, and dTTP, 0.1 mM dCTP, 100 μg/ml dactinomycin, and 200 μCi of[α-^{32}P]dCTP (3000 Ci/mmol; ICN Pharmaceuticals, Inc., Irvine, CA). After synthesis of the cDNA, the RNA was removed by incubation in 0.2M NaOH at 70°C for 20 min. The reaction was neutralized with HCl and the cDNA was ethanol precipitated in the presence of 2M ammonium acetate. The pellet was suspended in 200 μl of

TE (10 mM Tris HCl, pH 8.0, 1 mM EDTA) and added to the hybridization solution and filters. One of two probes was used to hybridize to each of the two replica filters; one was made from untreated FS-4 cells, and the other was made from FS-4 cells treated for 3h with TNF (10 ng/ml). After hybridization, the filters were washed in 2 x SSC (1 x SSC is 0.15M NaCl plus 0.015M sodium citrate)-0.1% sodium dodecyl sulfate (SDS) at 65°C for 1 h with one or two changes. Filters ere exposed to Kodak XAR-5 film for 1-2 days with an intensifying screen at -70°C. Plaques that showed different intensities of the hybridization signal with the two probes were selected. these clones were subjected to one further round of differential screening, and the plaques were purified.

Subcloning of the cDNA Inserts and Cross Hybridization Studies

E. coli LE392 cells in soft 0.7% agarose were poured into 150 mm plates. The lambda clones were then spotted on the plates in a grid array. Four nitrocellulose filters were lifter from each plate, processed, and stored until use. To prepare cDNA inserts from plaque-purified recombinant clones, 10 ml of liquid lysate was clarified and digested with 2 µg of DNase I per ml to remove contaminated chromosomal DNA. Then 2 ml of 2.5% SDS-0.5M Tris HCl (pH9.5)-0.25M EDTA was added, and plates were incubated at 65°C for 15 min to lyse the phages. The solution was then cooled to room temperature before 2.0 ml of 10M ammonium acetate was added. The sample was chilled on ice for 20 min and centrifuged at 15,000 x g at 4°C for 10 min to obtain the DNA pellet. The pellet was suspended in 100 µl TE buffer containing 2 Mg RNase A (Boehringer) per ml, and cut with the restriction enzyme EcoRI. The cDNA insert was isolated and subcloned into the EcoRI site of an M13mp19 vector. The cDNA inserts to be used as probes for cross-hybridization and Northern (RNA) blot experiments were prepared from the recombinant M13 clones by restriction with EcoRI to minimize background. The probes were prepared earlier. The hybridization conditions ere essentially as described above for the differential screening experiments.

Northern Blot Analysis

Cytoplasmic RNA was fractionated on a 1% agarose gel containing formaldehyde and blotted onto Zeta-probe blotting membranes (Bio-Rad Laboratories, Richmond, CA). Cytoplasmic RNA was loaded at 10 μg/lane. Prehybridization and hybridization of Northern blots were performed as described (Lin, J.-X. et al., supra). Filters were probed with 32P-labeled cDNA insert from recombinant M13 clones and/or with 32P-labeled internal reference pHe7 insert. Northern blots were quantified with a laser densitometer.

Sequence Analysis

Single stranded DNA templates from recombinant M13 clones were prepared, and several hundred nucleotides from each end of the cDNA were determined by the dideoxynucleotide-chain termination method (Sanger, F. et al., Proc. Natl. Acad. Sci. USA 74:5463-5468 (1977)). The partial nucleotide sequences were compared with sequences entered in GenBank (release 60.0).

RESULTS

FS-4 cells were grown to confluence, then switched to medium with 0.25% fetal bovine serum (FBS) and incubated for 72 h at 37°C. The cells were then exposed to recombinant human TNF (10 ng/ml). Cytoplasmic RNA was isolated (Lin, J.-X. et al., J. Biol. Chem. 262:11908 (1987)) after a 3-h incubation with TNF. A 3 h incubation with TNF was chosen for the following reason. It is known that TNF induces an increase in the level of some mRNAs within 20-30 min in quiescent FS-4 cells, but some of these "early response" mRNAs are elevated only transiently, for 30-120 min (e.g., c-fos and c-myc mRNA; see Lin, J.-X. et al., supra). Although such immediate early response gene products may be important for turning on other genes, the fact that they are induced only transiently suggested that they are not the actual effector molecules responsible for the phenotypic changes induced by TNF. Therefore, a search was initiated for cDNAs corresponding to messages that are more stably elevated after TNF treatment.

Poly(A)+ RNA was isolated from the cytoplasmic RNA by and double-stranded cDNA was synthesized. The resulting cDNA library from TNF-treated FS-4 cells, consisting of 2 x 10^6 recombinant clones, was screened for TNF-inducible gene sequences by differential hybridization with [^{32}P] cDNA probes prepared from poly(A)+ RNA from control and from TNF-treated FS-4 cells. Plaques which gave a strong signal when probed with cDNA from TNF-treated cells, but not when probed with control cell cDNA, were picked as presumptive TNF-inducible genes.

Approximately 3 x 10^4 plaques were screened, and 47 were scored as clearly inducible after two rounds of screening. They were designated TSG 1-47 (TSG= "TNF-stimulated gene sequence"). To determine the number of different mRNAs represented among the TSG clones selected by differential screening, the inserts were tested for sequence homology by cross-hybridization. A total of 44 cloned cDNAs have been examined by cross-hybridization to each other. These experiments revealed a total of eight distinct, non-crossreacting cDNAs. As summarized in Table 2, below, some of the cDNAs were represented among the 44 clones with a high frequency (TSG-8 and TSG-14) while others were much less abundant (TSG-21, TSG-27 and TSG-37). The size of the corresponding mRNAs ranged from 0.8 to 4.5 kb.

TABLE 2

Abundance of Individual TSG cDNAs Among 44 TNF-specific cDNA Clones

cDNA	Abundance[a]	Approximate size of corresponding mRNA (kb)
TSG-1	6	1.6
TSG-6	6	1.5
TSG-8	11	1.1
TSG-12	3	4.5
TSG-14	13	2.3
TSG-21	1	2.4
TSG-27	2	2.4
TSG-37	2	0.8

[a] Each of the 44 cDNA inserts was isolated from the #gt10 vector and subcloned into the M13mp19 vector. Inserts from the M13 vector were [32p]-labeled by nick translation and hybridized with each of the 44 lambda cDNA clones. Cross-hybridization was taken as evidence that the cDNAs were derived from the same mRNA species.

EXAMPLE II

Kinetics of Induction of TNF-Induced mRNAs

To ascertain that the eight distinct TSG cDNAs isolated indeed correspond to mRNAs whose levels are up-regulated in FS-4 cells by TNF treatment, quiescent FS-4 cultures were treated with TNF (20 ng/ml) for different intervals ranging from 0.5 to 16 h, cytoplasmic RNA was isolated (Lin, J.-X. et al., supra) and mRNA levels corresponding to each of the eight cDNAs were quantitated by Northern blot analysis and densitometric scanning of the autoradiograms (Figures 1 and 2). The increase in mRNA levels ranged from about 3-fold (TSG-21) to over 100-fold (TSG-6 and TSG-8).

Three different patterns of mRNA stimulation were noted. The first pattern was characterized by an increase to peak levels by 2-4 h, followed by a gradual decrease in mRNA levels (TSG-1 and TSG-6). The second pattern showed a rapid increase of mRNA levels to a maximum by 1.5 to 4 h, followed by a plateau until at least 16 h (TSG-8, TSG-12, TSG6-14 and TSG-37). The third pattern was characterized by a possible initial decrease, followed by a slow gradual increase in mRNA levels throughout the 16-h observation period (TSG-21 and TSG-27).

EXAMPLE III

Partial Sequencing of TSG cDNAs

To determine whether the isolated cDNAs were homologous to previously identified genes, all eight cDNAs were partially sequenced (300-400 bp) and the sequences determined were checked against sequences available in GenBank. Sequences of four cDNAs (TSG-1, TSG-8, TSG-21 and TSG-27) were found to be identical to earlier identified genes. Of these, TSG-1 corresponded to the gene for ß-thromboglobulin-like protein 3-10C (Schmid, J. et al., J. Immunol. 139:250 (1987)), also known as IL-8. TSG-8 was identical to the recently cloned gene for "monocyte chemotactic and activating factor" (MCAF) (Matsushima, K. et al., Cytokine 1:2 (1989)). TSG-21 and TSG-27 were found to be identical to the collagenase and stromelysin genes, respectively, and TSG-37 was found to encode metallothionein II. The other three partial cDNA sequences showed no

significant homologies with known genes, indicating that they represented hitherto unidentified gene sequences.

Induction of IL-8 (=TSG-1) by TNF and by IL-1 was recently observed by others (Matsushima, K. et al., 1989, supra; J. Exp. Med. 167:1883 (1988)). IL-8, a neutrophil chemotactic factor, is structurally related to several members of a family of inflammatory cytokines that include platelet factor-4 (PF-4), the IFN-gamma-inducible protein IP-10, the PDGF-inducible gene JE, proteins termed MIP-1 and MIP-2, and GRO (Matsushima, K. et al., supra; Larsen, C.G. et al., Science 243:1464 (1989)). Most of these proteins appear to be chemotactic.

MCAF (=TSG-8) induction in human fibroblasts by TNF and IL-1 has been recently described (Matsushima, K. et al., supra). Interestingly, MCAF shows significant amino acid sequence similarity (21%) with IL-8, and they both have four cysteines at similar positions.

Collagenase (TSG-21) was also reported earlier to be TNF-inducible in synovial cells and fibroblasts (Dayer et al., supra). It is very likely that the ability of TNF to induce collagenase is related to TNF's role in tissue remodeling during inflammation. While the induction of stromelysin by TNF has not been reported, stromelysin mRNA was recently shown to be inducible by IL-1 (Quinones, S.et al., J. Biol. Chem. 264:8339 (1989)). Like collagenase, stromelysin is a collagen-degrading metalloproteinase, and both can also degrade fibronectin, laminin and cartilage proteoglycans. Both collagenase and stromelysin are thought to be important

in the increased extracellular matrix degradation occurring in rheumatoid arthritis.

Finally, metallothionein II (MT-II) has been shown to be inducible by various stresses, including heavy metal challenge, injection of lipopolysaccharide as well as by cytokines including interferons and IL-1) (Karin, M., Cell 41:9-10 (1985)). In addition to its ability to bind heavy metal ions, MT-II may also act as a scavenger of free radicals released by activated macrophages and neutrophils during an inflammatory response. MT-II induction would thus serve a protective role in the prevention of tissue injury (Thornalley, P.J., Biochim. Biophys. Acta 827:36-44 (19__).

It is significant that all five TSG cDNAs identified by sequencing correspond to genes coding for proteins important in the inflammatory process. These results support the utility of the present approach of cloning TNF-inducible cDNAs from human fibroblasts in the identification of novel genes with important functions in immune responses and inflammation.

EXAMPLE IV

Patterns of TSG-14 mRNA Induction by Different Cytokines and Other Agents

Table 3 provides summarizes data of experiments which measured levels of TSG-14 mRNA in FS-4 cells exposed to various agents using Northern blot analysis. TSG-14 was inducible by the protein synthesis inhibitor, cycloheximide, as was the case for other TNF-induced mRNAs, TSG-8 and TSG-12. The addition of cycloheximide did not TSG-14 mRNA inducibility by TNF, suggesting that the increase in the mRNA levels was the result of a direct action of TNF, not requiring a protein intermediate. TSG-14 mRNA was not inducible by IFN-ß of

IFN-gamma. TSG-14 mRNA was inducible by IL-1, by the double-stranded RNA poly(I):poly(C), and by the phorbol ester 12-O-tetradecanoyl phorbol 13-acetate (TPA).

Table 3

INDUCTION OF TSG-14 mRNA

Stimulus	Conc.	Relative Increase in TSG-14 mRNA
Tumor necrosis factor (TNF)	20 ng/ml	+++
Cycloheximide	10 µg/ml	+
TNF + cycloheximide		+++
Interleukin-1	1 ng/ml	+++
Interferon-ß	500 U/ml	0
Interferon-gamma	100 U/ml	0
TNF + IFN-ß		+++
TNF + IFN-gamma		+++
Epidermal growth factor	25 ng/ml	+
Platelet derived growth factor	5 ng/ml	+
Transfroming growth factor-ß	2 ng/ml	0
Poly(I):Poly(C)	50 µg/ml	++
Phorbol ester (TPA)	100 ng/ml	+
A23187	1 µM	0
Forskolin	10 µM	0
dibutyryl cyclic AMP	100 µM	0
Isobutyl methylxanthine	100 µM	0

The results shown are a summary of northern blot experiments in which total cellular RNAs were extracted from FS-4 cells treated for 2 hrs with the indicated concentration of agent or agents. Results reflect the relative increase in TSG-14 mRNA levels over control.

Epidermal growth factor (EGF) and platelet derived growth factor (PDGF) each stimulated a moderate induction of TSG-14 mRNA. Transforming growth factor-ß (TGF-ß), the calcium ionophore, A23187, dibutyryl cyclic AMP and the phosphodiesterase inhibitor, isobutyl methylxanthine, were ineffective in inducing TSG-14 mRNA.

EXAMPLE V

Complete DNA Sequence of TSG-14

TSG-14 was cloned into M13mp18 and sequenced. The cDNA sequence of TSG-14 was found to consist of 1837 base pairs (SEQ ID NO:1; see Figure 4). Complete sequence of the cDNA was derived by sequencing several shorter overlapping fragments. Analysis of the sequence revealed three open reading frames, but only one of these appears to be the correct reading frame, based on several considerations. First, this ORF contains a short 5' 73-bp untranslated region, which is consistent with the character of most mRNAs. Second, the initiation codon for this ORF is in the context of a consensus start region. Finally, the 5' coding region encodes a 17 amino acid-long highly hydrophobic region (Figures 3 and 4), with characteristics of a cleavable signal peptide sequence.

The ORF of TSG-14 consists of 534 nucleotides, encoding a protein 178 amino acids long (SEQ ID NO:2; see Figure 4). The transcript contains a very long 3' untranslated region, a feature not unusual among cytokines. Furthermore, 2 polyadenylation sites are present at the extreme 3' end of the sequence. Most interestingly, the 3' noncoding region of the TSG-14 sequence contains a region of greater than 70% homology with the 3' region of another cytokine, namely IL-1ß, based on information derived from the Genbank database. Taken together, these features indicate that TSG-14 is a novel, biologically active cytokine.

EXAMPLE VI

TSG-14 Amino Acid Sequence and Protein Structure

The predicted sequence of the TSG-14 protein has several remarkable features. No potential N-glycosylation sites are present. Interestingly, while the mature protein only one lysine residue is found in the entire protein. Five cysteine residues are present in the mature protein, indicating inter- or intra-molecular disulfide bridges.

A hydrophobicity plot, using the algorithm of Kyte and Doolitle (supra), predicts the presence of a highly hydrophobic sequence at the N-terminus (Figure 5). Indeed, the first 17 amino acids are quite hydrophobic in character, suggesting that this stretch represents a cleavable signal sequence or membrane anchor.

Plotting TSG-14 as a function of the isoelectric points of the individual amino acid (see Figure 6) residues revealed that the N-terminal half of the protein is strongly acidic. Indeed, within a 50-amino acid region there are 15 acidic amino acids, whereas only 4 basic residues are found in the same stretch. The rest of the protein has a slight excess of basic over acidic amino acids. The net charge of this protein is acidic (calculated pI=4.51); the protein is therefore highly soluble.

Analysis of the TSG-14 protein secondary structure revealed that several regions are expected to be highly antigenic (based on the relative antigenicity of individual amino acids in the context of neighboring residues). This property is of benefit in the preparation of antibodies to TSG-14 (see below). A homology search based on the predicted amino acid sequence was conducted against other sequences in the NBRF protein database (release 18.0). No significant homology was found with any other protein, indicating that TSG-14 indeed represents a novel protein.

EXAMPLE VII

Preparation of Bacterial Fusion-Protein and Expression of Protein

To express a bacterial fusion protein of TSG-14, a TSG-14 insert containing a Sau3A site at its 5' end was used. A Sau3A site at the 3' end of the insert was generated when an EcoRI fragment of TSG-14 was inserted into the pTZ19 vector system, and subsequently cut with the Sau3A enzyme. This insert was then ligated into a unique BamHI site within the coding region of the trpE protein of the pATH2 vector, generating the pATH2-TSG-14 expression vector. (See Figure 7

for depiction of vector.) Expression of the fusion protein was under the control of the Trp promoter.

Expression plasmid pATH2--TSG-14 was transferred into competent E. coli JM101 cells. Transformed cells in M9 medium containing 2% Casamino acids, 20 μg/ml of L-tryptophan, and 150 μg/ml of ampicillin were grown to a density of A_{600} =0.5 (absorbance at 600 nm). To induce synthesis of the fusion protein, cells were pelleted and resuspended in prewarmed L-tryptophan-free medium. After an additional 1-hour incubation, 20 μg/ml of 3-β-indoleacrylic acid was added and the incubation was continued for an additional 24 hours. Figure 8 shows that protein of the expected size (approximately 60 kDa) was in fact induced following addition of 3-β-indoleacrylic acid. A truncated trpE-TSG-14 fusion protein resulting from cutting the pATH2-TSG-14 vector with PstI, having a molecular weight of about 44 kDa, is also shown.

EXAMPLE VIII

Purification of TSG-14 Fusion Protein

Purification of the fusion protein described above is performed essentially as described by Strebel et al. (J. Virol. 57:983-991 (1985)). Cells from a 1L culture are pelleted and washed with TEN (10 mM Tris-HCL, pH 8.0, 1 mM EDTA, 0.5M NaCl), lysed with lysozyme (5 mg/ml) and finally broken by sonication. Insoluble material is recovered by centrifugation (30 min, 20,000 x g) and extracted sequentially with 20 ml of 3M urea and 5 ml of 7M urea each for 30 min at 37°C. The 7M urea extract containing the fusion protein is further purified by preparative SDS-PAGE. After electrophoresis the fusion protein is excised from the gel, electroeluted and concentrated as needed. The purity of the electroeluted fusion protein is checked on analytical gels. After the second round of electroelution, highly purified fusion protein is obtained with no detectable E. coli protein bands on SDS-PAGE.

EXAMPLE IX

Expression of TSG-14 in Various Cells

The biological functions of TSG-14 can be studied by expression of TSG-14 cDNA in a cell line which does not respond to TNF by the induction of TSG-14 mRNA. The inducibility of TSG-14 mRNA by TNF was thus examined in various cell lines by Northern blot analysis. Included in this study were a number of of SV40-transformed cell lines and tumor cell lines. The results are shown in Table 4.

TABLE 4

Induction of TSG-14 mRNA by TNF

Cell Line	Control	TNF (20ng/ml, 4hr)
Normal Cells		
FS-4	-	+
HUVEC	-	+
SV40-Transformed Cell Lines		
FS-4 SV2	+/-	++
FS-4 SV3	+/-	++
FS-4 SV4	+/-	++
WI-38 VA	-	+/-
GM637	-	+
Tumors		
A549	-	-
A673	-	-
Colo205	-	-
HT29	-	-
SK-Mel-19	-	-
U937	-	-

The data represent a summary of northern blots of TSG-14 mRNA in normal, SV40-transformed, and tumor cell lines. HUVEC: human umbilical vein endothelial cells; SV2, SV3 and SV4: FS-4 cells transformed with SV40 large T antigen; WI-38: SV40-transformed lung fibroblasts; GM637: SV40-transformed skin fibroblasts; A549: lung carcinoma line; A637: rhabdomyosarcoma line; Colo205: colon adenocarcinoma line; HT29: colon adenocarcinoma line; SK-Mel-19: melanoma line; U937: promonocytic leukemia line.

Thus, TSG-14 mRNA is expressed in response to TNF in some cells only. No expression was observed in the six tumor lines

analyzed, even in the presence of TNF. Interestingly, the levels of TSG-14 mRNA were higher in FS-4 cells transfected with the large T-antigen of SV40, which transforms these cells, compared to normal, nontransformed counterparts. This is in contrast to another TNF-induced protein, TSG-6, where TNF induction of TSG-6 mRNA was significantly decreased in FS-4 cells transfected with SV40 large T antigen. Thus, the degree of "oncogenic" transformation has a differential effect on the inducibility of TSG-14 versus TSG-6 mRNA.

EXAMPLE X

Transfection and Expression of TSG-14 cDNA in Cell Lines

Mammalian expression vectors of TSG-14 are constructed using pSV2 (Mulligan, R.C. et al., Science 209:1423 (1980)) as a constitutive expressor and pMAMneo (Sardet, C. et al., Cell 56:271 (1989)) as a dexamethasone-inducible expressor, respectively. These constructs are used to transfect cells in which TSG-14 is not inducible by TNF, namely, the A549 cell line, using $CaPO_4$ precipitation. In the case of stable transfection with a pSV2-TSG-14 vector, pRSVneo (Gorman, C. et al., Science 221:551 (1983)), which confers resistance to the antibiotic G418, is cotransfected.

Stable transfectants are selected in a G418 containing medium, and tested for the expression of TSG-14 cDNA by northern blot analysis. Transfectants express TSG-14 mRNA in the absence of TNF. The major band in a northern blot is of the same size as the band corresponding to TSG-14 mRNA induced by TNF in FS-4 cells.

EXAMPLE XI

Preparation of Polyclonal Antiserum and Purification of Anti-TSG-6 Antibodies by Immunoaffinity Chromatography

Rabbits are first immunized with about 200 μg of the TrpE/TSG-14 fusion protein suspended in Freund's complete adjuvant and are boosted at intervals of 2-3 weeks with the same amount of protein in Freund's incomplete adjuvant. All injections are performed subcutaneously, except for the final boost which is intravenous. Rabbits are bled about six days after immunizations. Sera are analyzed by immunoblotting according to Strebel et al. (J. Virol. 57:983-991 (1985)).

To obtain antibodies specific for TSG-14 domains of the fusion protein, the antiserum is subjected to purification on an immunoaffinity matrix to which a a second TSG-14 fusion protein, for example, a MS2/TSG-14 fusion protein is coupled. Such a fusion protein is produced using the plasmid pEX34A, a derivative of pEX29 (Klinkert, M. et al., Infec. Immun. 49:329-335 (1985)), which permits the production of foreign proteins fused to the N-terminal part of the MS2 polymerase and controlled by the temperature-inducible lambda PL promoter.

The immunoaffinity chromatography matrix is prepared as follows. Five mg of purified MS2/TSG-14 fusion protein is dialyzed extensively against 0.5M NaCl. Three ml of EAH-Sepharose 4B (Pharmacia) is washed extensively with 0.5M NaCl and the purified MS2/TSG-6 fusion protein is added. The pH is adjusted to 4.5 and 40 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Aldrich Chemical Co.) dissolved in 1 ml distilled water is added dropwise while constantly stirring. Thereafter, the pH is readjusted to 4.5 and the coupling reaction is allowed to proceed overnight under constant stirring. Acetic acid (200 μl) is added for another 4 hr to block the remaining amino groups on the matrix. Finally, the matrix material is washed several times alternatively with 0.1M acetate buffer, pH 4.0, 0.5M NaCl, and 0.1M sodium bicarbonate buffer, pH 8.3, 0.5M NaCl, and suspended in Tris-buffered saline for storage.

For immunoaffinity chromatography, 0.5 ml of MS2/TSG-14 Sepharose is equilibrated with Tris-buffered saline (20 mM Tris, 0.5M NaCl, pH 7.5) containing 0.05% Tween-20 (TTBS). One ml of antiserum raised against the TrpE/TSG-14 fusion protein is mixed with 0.5 ml MS2/TSG-14 Sepharose and 0.5 ml TTBS, and the mixture incubated in a cryotube at 4°C overnight under constant rotation. The suspended solid phase matrix material is transferred to a centrifuge tube and washed with 10 ml TTBS. Thereafter, the sediment is transferred to an Eppendorf tube, centrifuged (14,000 rpm, 2 min.) and the supernatant carefully removed. One ml 0.1M glycine-HCl buffer, pH 2.5, is added and the gel is shaken for 2 min. After further centrifugation, the supernatant is immediately neutralized with solid Tris. This results in the production of a specific antiserum for TSG-14.

EXAMPLE XII

Detection of Natural and Recombinant TSG-14 Protein from TNF-Treated FS-4 Cells and Cells Transfected with a TSG-14 Expression Vector

Experiments are conducted to localize TSG-14 protein in the supernatants or extracts of either (a) FS-4 cells treated with TNF for 9 hours, or (b) cells transfected with TSG-14 cDNA.

After the cells (either normal FS-4 or TSG-14-transfected cells) are grown to confluence, the medium is removed and replenished with serum-free medium. The FS-4 cells are either stimulated with 20 ng/ml TNF or left untreated. After 7 to 24 hours, the medium is collected and concentrated up to 100-fold in an Amicon apparatus. Cell pellets are also collected and dissolved in SDS-PAGE sample buffer. Samples are subjected to Western blot analysis with the aid of immunopurified antiserum against TSG-14 protein.

The major band which was specifically recognized by the immunopurified antiserum is detected in the culture supernatants of both TNF-treated FS-4 cells and TSG-14 transfected cells, but not of control cells. This protein migrates at an apparent molecular weight of 16-19 kDa on SDS-

PAGE. The estimated molecular weight of the secreted TSG-14 protein,. based on the primary sequence, is approximately 17.5 kDa.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lee, Tae Ho
Lee, Gene W.
Vilcek, Jan

(ii) TITLE OF INVENTION: Cytokine-Induced Protein, TSG-14, DNA Coding Therefor and Uses Thereof

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Browdy and Neimark
(B) STREET: 419 Seventh Street, NW
(C) CITY: Washington
(D) STATE: DC
(F) ZIP: 20004

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.24

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Livnat, Shmuel
(B) REGISTRATION NUMBER: 33,949
(C) REFERENCE/DOCKET NUMBER: VILCEK=2

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202)628-5197
(B) TELEFAX: (212)737-3528

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1836 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(G) CELL TYPE: Fibroblast
(H) CELL LINE: FS-4

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 73..606
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AATTCGCGCT CAACTCAGCT CACTGAGAGT CTCTCCCGCC AGCTGTGGAA AGAACTTTGC   60

GTCTCTCCAG CA ATG CAT CTC CTT GCG ATT CTG TTT TGT GCT CTC TGG       108
                Met His Leu Leu Ala Ile Leu Phe Cys Ala Leu Trp
                  1               5                  10

TCT GCA GTG TTG GCC GAG AAC TCG GAT GAT TAT GAT CTC ATG TAT GTG     156
Ser Ala Val Leu Ala Glu Asn Ser Asp Asp Tyr Asp Leu Met Tyr Val
        15                  20                  25

AAT TTG GAC AAC GAA ATA GAC AAT GGA CTC CAT CCC ACT GAG GAC CCC     204
Asn Leu Asp Asn Glu Ile Asp Asn Gly Leu His Pro Thr Glu Asp Pro
    30                  35                  40

ACG CCG TGC GAC TGC GGT CAG GAG CAC TCG GAA TGG GAC AAG CTC TTC     252
Thr Pro Cys Asp Cys Gly Gln Glu His Ser Glu Trp Asp Lys Leu Phe
45                  50                  55                  60

ATC ATG CTG GAG AAC TCG CAG ATG AGA GAG CGC ATG CTG CTG CAA GCC     300
Ile Met Leu Glu Asn Ser Gln Met Arg Glu Arg Met Leu Leu Gln Ala
                65                  70                  75

ACG GAC GAC GTC CTG CGG GGC GAG CTG CAG AGG CTG CGG GAG GAG CTG     348
Thr Asp Asp Val Leu Arg Gly Glu Leu Gln Arg Leu Arg Glu Glu Leu
            80                  85                  90

GGC CGG CTC GCG GAA AGC CTG GCG AGG CCG TGC GCG CCG GGG GCT CCC     396
Gly Arg Leu Ala Glu Ser Leu Ala Arg Pro Cys Ala Pro Gly Ala Pro
        95                  100                 105

GCA GAG GCC AGG CTG ACC AGT GCT CTG GAC GAG CTG CTG CAG GCG ACC     444
Ala Glu Ala Arg Leu Thr Ser Ala Leu Asp Glu Leu Leu Gln Ala Thr
    110                 115                 120

CGC GAC GCG GGC CGC AGG CTG GCG CGT ATG GAG GGC GCG GAG GCG CAG     492
Arg Asp Ala Gly Arg Arg Leu Ala Arg Met Glu Gly Ala Glu Ala Gln
125                 130                 135                 140

CGC CCA GAG GAG GCG GGG CGC GCC CTG GCC GCG GTG CTG AGG AGC TGC     540
Arg Pro Glu Glu Ala Gly Arg Ala Leu Ala Ala Val Leu Arg Ser Cys
                145                 150                 155

GGC GAC GCG AGC CGA CCT GCA CGC GGT GCA GGG CTG GGC TGC CCG GAG     588
Gly Asp Ala Ser Arg Pro Ala Arg Gly Ala Gly Leu Gly Cys Pro Glu
```

160 165 170

CTG GCT GCC GGC AGG TTG TGAAACAGCT ATTTTATTCC CAATGCGTTC 636
Leu Ala Ala Gly Arg Leu
175

CAAGAAGATT TTTGGAAGCG TGCATCCAGT GAGACCTTTG AGGCTTGAGT CTTTTAGTGC 696

CTGCATTTGG GTCAAAGCCA CAGATGTATT AAACAAAACC ATCCTGTTTT CCTATGGCAC 756

AAAGAGGAAT CCATATGAAA TCCAGCTGTA TCTCAGCTAC CAATCCATAG TGTTTGTGGT 816

GGGTGGAGAG GAGAACAAAC TGGTTGCTGA AGCCATGGTT TCCCTGGGAA GGTGGACCCA 876

CCTGTGCGGC ACCTGGAATT CAGAGGAAGG GCTCACATCC TTGTGGGTAA ATGGTGAACT 936

GGCGGCTACC ACTGTTGAGA TGGCCACAGG TCACATTGTT CCTGAGGGAG GAATCCTGCA 996

GATTGGCCAA GAAAAGAATG GCTGCTGTGT GGGTGGTGGC TTTGATGAAA CATTAGCCTT 1056

CTCTGGGAGA CTCACAGGCT TCAATATCTG GGATAGTGTT CTTAGCAATG AAGAGATAAG 1116

AGAGACCGGA GGAGCAGAGT CTTGTCACAT CCGGGGGAAT ATTGTTGGGT GGGGAGTCAC 1176

AGAGATCCAG CCACATGGAG GAGCTCAGTA TGTTTCATAA ATGTTGTGAA ACTCCACTTG 1236

AAGCCAAAGA AAGAAACTCA CACTTAAAAC ACATGCCAGT TGGGAAGGTC TGAAAACTCA 1296

GTGCATAATA GGAACACTTG AGACTAATGA AAGAGAGAGT TGAGACCAAT CTTTATTTGT 1356

ACTGGCCAAA TACTGAATAA ACAGTTGAAG GAAAGACATT GGAAAAAGCT TTTGAGGATA 1416

ATGTTACTAG ACTTTATGCC ATGGTGCTTT CAGTTTAATG CTGTGTCTCT GTCAGATAAA 1476

CTCTCAAATA ATTAAAAAGG ACTGTATTGT TGAACAGAGG GACAATTGTT TTACTTTTCT 1536

TTGGTTAATT TTGTTTTGGC CAGAGATGAA TTTTACATTG GAAGAATAAC AAAATAAGAT 1596

TTGTTGTCCA TTGTTCATTG TTATTGGTAT GTACCTTATT ACAAAAAAAA TGATGAAAAC 1656

ATATTTATAC TACAAGGTGA CTTAACAACT ATAAATGTAG TTTATGTGTT ATAATCGAAT 1716

GTCACGTTTT TGAGAAGATA GTCATATAAG TTATATTGCA AAAGGGATTT GTATTAATTT 1776

AAGACTATTT TTGTAAAGCT CTACTGTAAA TAAAATATTT TATAAAACTA AACGGAATTC 1836

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 178 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Leu Leu Ala Ile Leu Phe Cys Ala Leu Trp Ser Ala Val Leu
1 5 10 15

Ala Glu Asn Ser Asp Asp Tyr Asp Leu Met Tyr Val Asn Leu Asp Asn
20 25 30

Glu Ile Asp Asn Gly Leu His Pro Thr Glu Asp Pro Thr Pro Cys Asp
35 40 45

Cys Gly Gln Glu His Ser Glu Trp Asp Lys Leu Phe Ile Met Leu Glu
50 55 60

Asn Ser Gln Met Arg Glu Arg Met Leu Leu Gln Ala Thr Asp Asp Val
65 70 75 80

Leu Arg Gly Glu Leu Gln Arg Leu Arg Glu Glu Leu Gly Arg Leu Ala
85 90 95

Glu Ser Leu Ala Arg Pro Cys Ala Pro Gly Ala Pro Ala Glu Ala Arg
100 105 110

Leu Thr Ser Ala Leu Asp Glu Leu Leu Gln Ala Thr Arg Asp Ala Gly
115 120 125

Arg Arg Leu Ala Arg Met Glu Gly Ala Glu Ala Gln Arg Pro Glu Glu
130 135 140

Ala Gly Arg Ala Leu Ala Ala Val Leu Arg Ser Cys Gly Asp Ala Ser
145 150 155 160

Arg Pro Ala Arg Gly Ala Gly Leu Gly Cys Pro Glu Leu Ala Ala Gly
165 170 175

Arg Leu

WHAT IS CLAIMED IS:

1. The tumor-necrosis factor-induced protein molecule TSG-14, or a functional derivative thereof, wherein, when said molecule is one which naturally occurs, said molecule is substantially free of other proteins or glycoproteins with which it is natively associated.

2. The molecule of claim 1 having an apparent molecular weight of about 16 to about 19 kDa under denaturing conditions.

3. The molecule of claim 1 having the amino acid sequence SEQ ID NO:2, or a functional derivative thereof.

4. A DNA molecule encoding TSG-14 or a functional derivative thereof, wherein, when said DNA molecule naturally occurs, it is substantially free of the adjoining nucleotide sequences with which it is natively associated.

5. A DNA molecule encoding a human TSG-14 protein according to claim 4, substantially free of other human nucleotide sequences.

6. A DNA molecule according to claim 4 which is cDNA.

7. A DNA molecule according to claim 4 which is genomic DNA.

8. A DNA molecule according to claim 4 having the nucleotide sequence SEQ ID NO:1.

9. A DNA molecule according to claim 4 which is an expression vehicle.

10. A DNA molecule according to claim 9 wherein said vehicle is a plasmid.

11. A prokaryotic host transformed with the molecule of claim 9.

12. A host according to claim 10 which is a bacterium.

13. A eukaryotic host transfected with the molecule of claim 9.

14. A host according to claim 13 which is a yeast cell or a mammalian cell.

15. A process for preparing the human TSG-14 protein molecule, or a functional derivative thereof, comprising:

(a) culturing a host cell capable of expressing said protein under culturing conditions,

(b) expressing said protein or functional derivative; and

(c) recovering said protein or functional derivative from said culture.

16. A process according to claim 15 wherein said host is prokaryotic.

17. A process according to claim 15 wherein said host is eukaryotic.

18. An antibody specific for the protein of claim 1.

19. The antibody of claim 18 which is monoclonal.

20. A method for detecting the presence of TSG-14 protein in a biological sample, comprising:

(a) contacting said biological sample that is suspected of containing said TSG-14 protein with a molecule capable of binding to said protein; and

(b) detecting any of said molecule bound to said protein.

21. The method of claim 20, wherein said molecule is an antibody or a fragment thereof.

22. The method of claim 21 wherein said antibody is a monoclonal antibody

23. A method for detecting the presence of nucleic acid encoding a normal or mutant TSG-14 protein in a subject comprising:

(a) contacting a cell obtained from said subject, an extract thereof, or a culture supernatant thereof, with an oligonucleotide probe encoding at least a portion of said normal or mutant TSG-14 under hybridizing conditions; and

(b) measuring the hybridization of said probe to the nucleic acid of said cell, thereby detecting the presence of said nucleic acid.

24. The method of claim 23, additionally comprising, before step (a):

(c) selectively amplifying the amount of DNA of said cell encoding said TSG-14 protein.

25. A method for measuring induction of expression of TSG-14 in a cell, comprising:

(a) contacting said cell with a substance capable of inducing expression of TSG-14;

(b) measuring the amount of mRNA encoding TSG-14 in said cell by hybridization with an oligonucleotide probe encoding at least a portion of TSG-14, under hybridizing conditions; and

(c) comparing the amount of TSG-14-encoding mRNA in said cell with the amount of TSG-14-encoding mRNA in said cell not contacted with said substance,

wherein an increase in the amount of said mRNA indicates that said induction has occurred.

26. A method for measuring induction of expression of TSG-14 in a cell, comprising:

(a) contacting said cell with a substance capable of inducing expression of TSG-14;

(b) measuring the amount of TSG-14 protein in an extract or supernatant of said cell using the method according to claim 20.

(c) comparing the amount of TSG-14 protein in said cell extract or supernatant with the amount of TSG-protein in the extract or supernatant of said cell not contacted with said substance,

wherein an increase in the amount of the TSG-14 protein indicates that said induction has occurred.

27. A method for identifying a compound capable of inducing the expression of TSG-14 in a cell, comprising:

(a) contacting said cell with said compound; and

(b) measuring the induction of TSG-14 mRNA using the method according to claim 24, wherein said inducing substance is said compound,

thereby identifying said compound.

28. A method for identifying a compound capable of inducing the secretion of TSG-14 protein from a cell, comprising:

(a) contacting said cell with said compound;

(b) measuring the presence of TSG-14 protein in the supernatant of said cell using the method according to claim 20,

thereby identifying said compound.

FIG. 1A

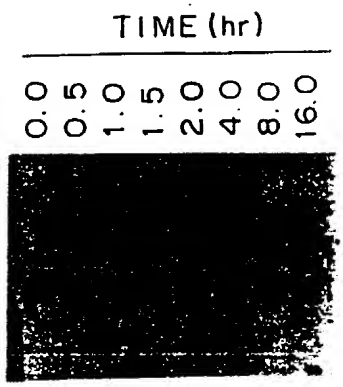


FIG. 1B

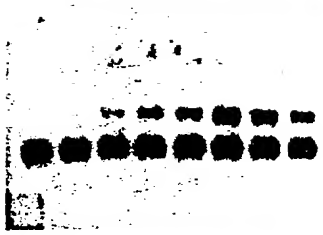


FIG. 1C

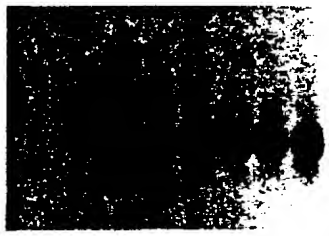


FIG. 1D

FIG. 1E

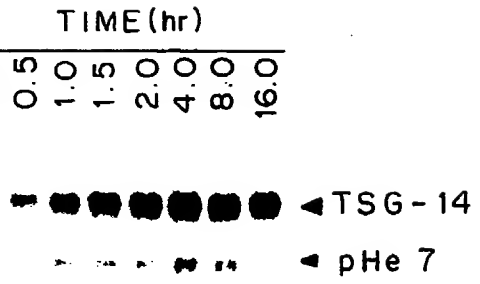


FIG. 1F

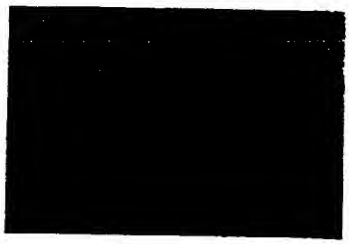


FIG. 1G

FIG. 1H

FIG. 2A

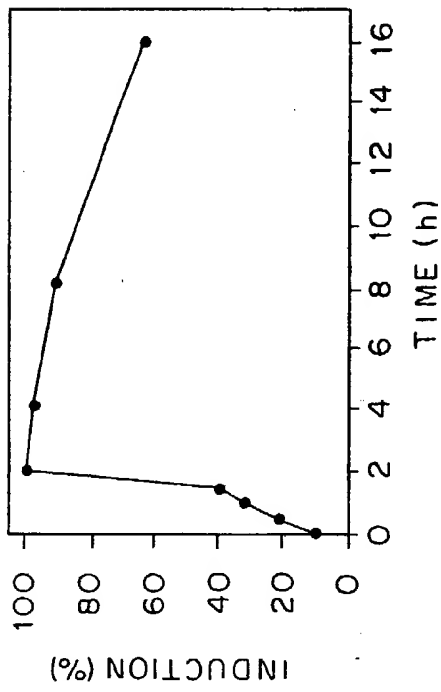


FIG. 2B

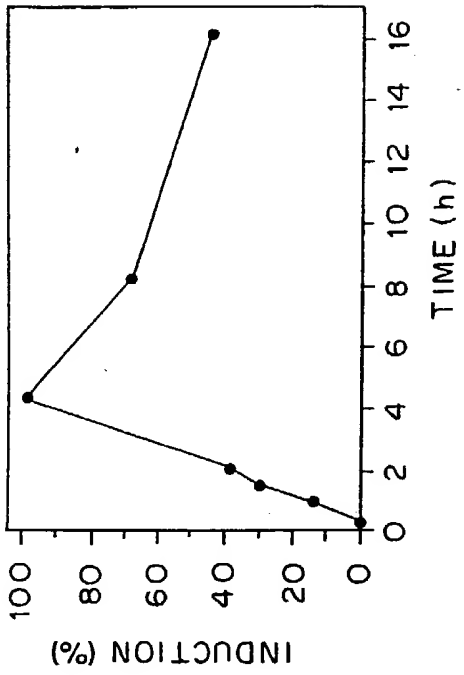


FIG. 2C

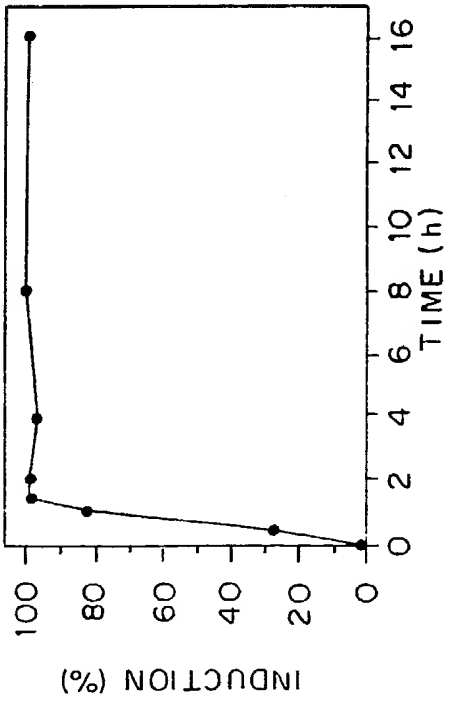
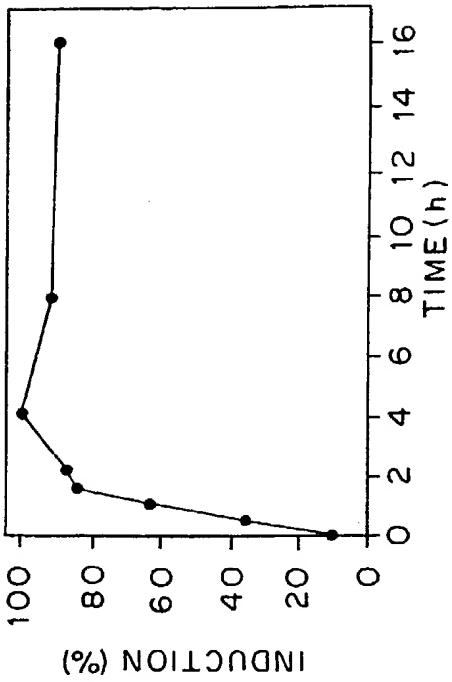


FIG. 2D



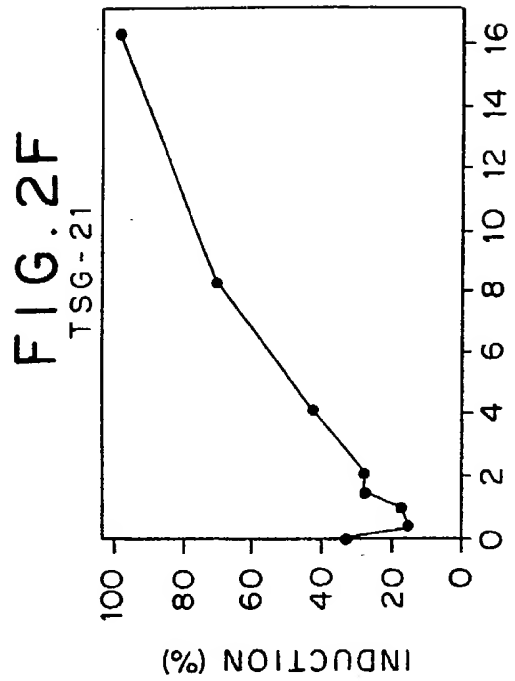


FIG.2F
TSG-21
INDUCTION (%)
TIME (h)
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16

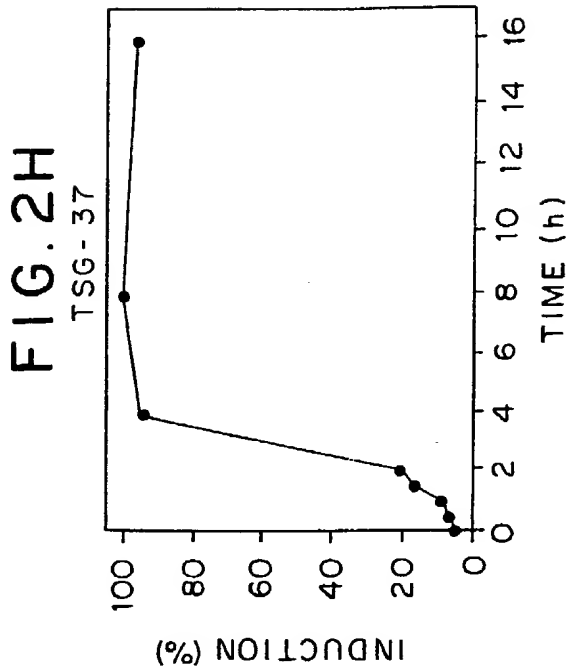


FIG.2H
TSG-37
INDUCTION (%)
TIME (h)
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16

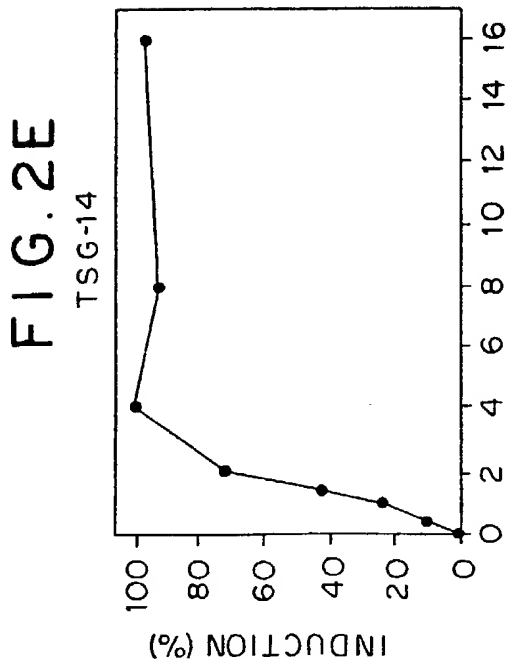


FIG.2E
TSG-14
INDUCTION (%)
TIME (h)
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16

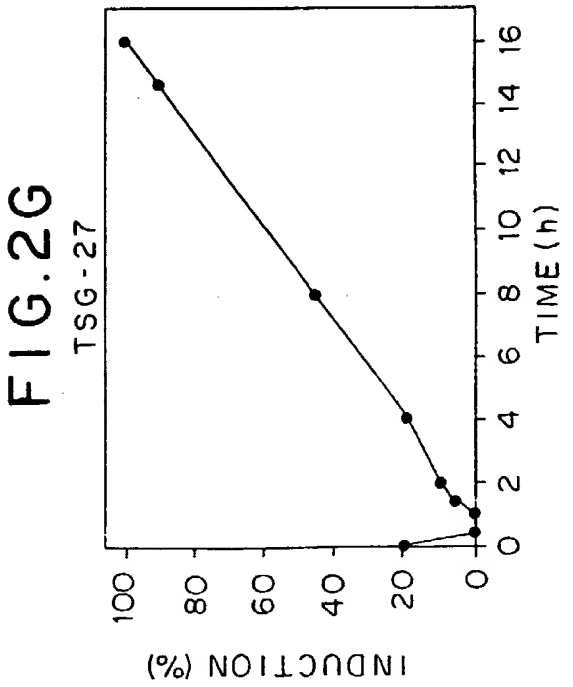


FIG.2G
TSG-27
INDUCTION (%)
TIME (h)
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16

FIG. 3A

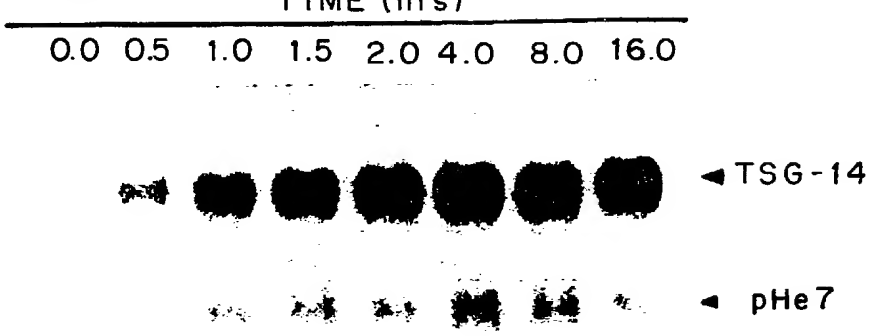

TIME (hrs)
0.0 0.5 1.0 1.5 2.0 4.0 8.0 16.0
TSG-14
pHe7

FIG. 8

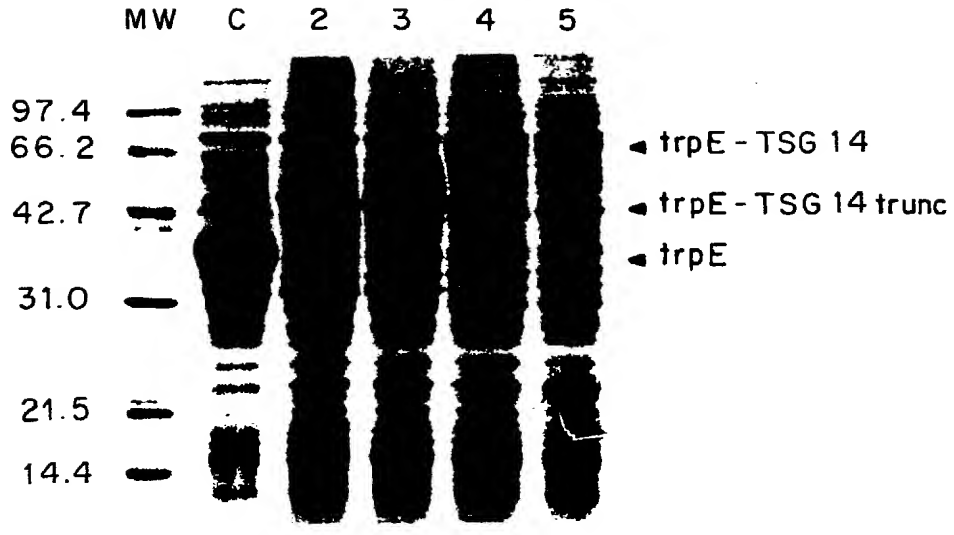

MW C 2 3 4 5
97.4
66.2
42.7
31.0
21.5
14.4
trpE - TSG 14
trpE - TSG 14 trunc
trpE

FIG. 3b

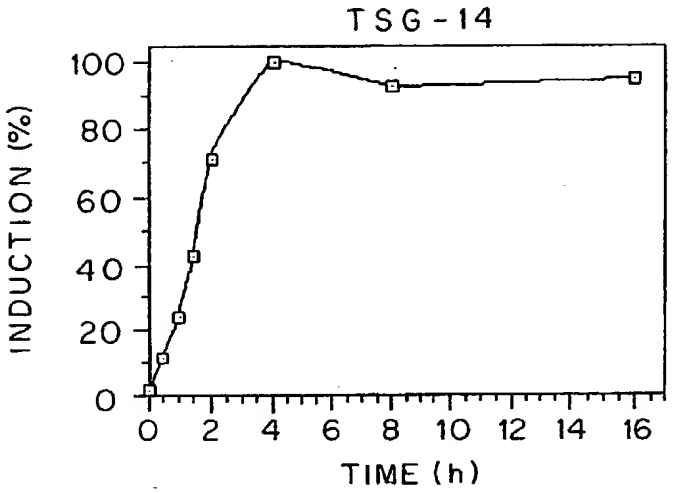

TSG-14
INDUCTION (%)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16
TIME (h)

FIG. 7

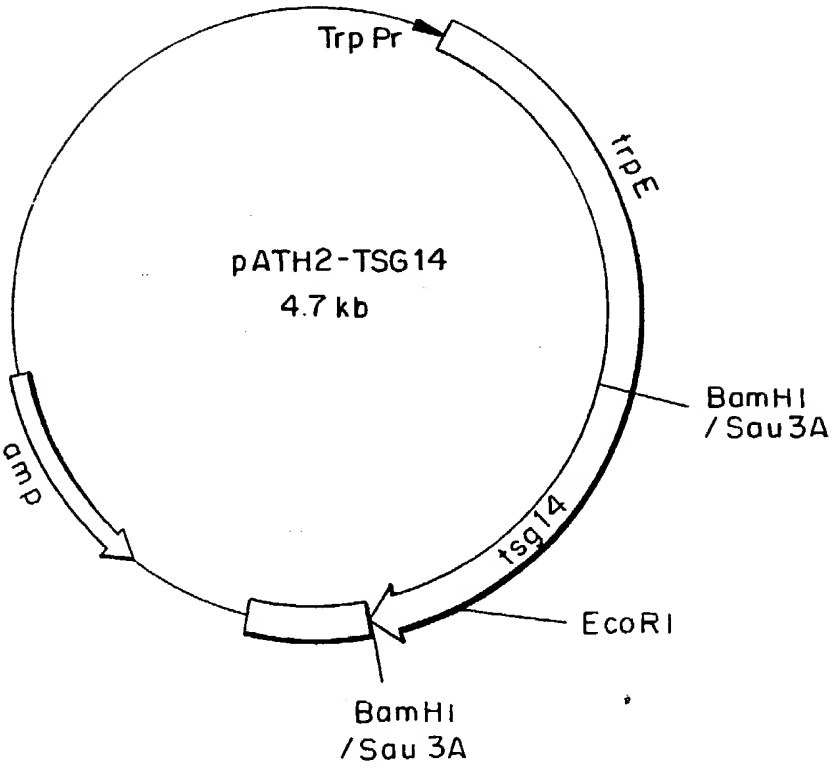

Trp Pr
trpE
pATH2-TSG14
4.7 kb
BamHI
/Sau3A
amp
tsg14
EcoRI
BamHI
/Sau 3A

FIG. 4A

```
1
AATTCGCGCTCAACTCAGCTCACTGAGAGTCTCTCCCGCCAGCTGTGGAAAGAACTTTGCGTCTCTCCACGAATGCATCTCCTTGCGATTCTG
                                                                MetHisLeuLeuAlaIleLeu

94
TTTTGTGCTCTCTGGTCTGCAGTGTTGGCCGAGAACTCGGATGATTATGATCTCATGTATGTGAATTTGGACAACGAAATAGACAATGGACTC
PheCysAlaLeuTrpSerAlaValLeuAlaGluAsnSerAspAspTyrAspLeuMetTyrValAsnLeuAspAsnGluIleAspAspGlyLeu

187
CATCCCACTGAGGACCCCACGCCGTGCGACTGCGGTCAGGAGCACTCGGAATGGCACAAGCTCTTCATCATGCTGGAGAACTCGCAGATGAGA
HisProThrGluAspProThrProCysAspCysGlyGlnGluHisSerGluTrpAspLysLeuPheIleMetLeuGluAsnSerGlnMetArg

280
GAGCGCATGCTGCTGCAAGCCACGGACGACGTCCTGCGGGGCGAGCTGCAGAGGCTGCGGGAGGACCTGGGCCGGCTCGCGGAAAGCCTGGCG
GluArgMetLeuLeuGlnAlaThrAspAspValLeuArgGlyGluLeuGlnArgLeuArgGluGluLeuGlyArgLeuAlaGluSerLeuAla

373
AGGCCGTGCGCGCCGGGGGCTCCCGCAGAGGCCAGGCTGACCAGTGCTCTGGACGAGCTGCTGCAGGCGACCCGCGACGCGGGCCGCAGGCTG
ArgProCysAlaProGlyAlaProAlaGluAlaArgLeuThrSerAlaLeuAspGluLeuLeuGlnAlaThrArgAspAlaGlyArgArgLeu

466
GCGCGTATGGAGGGCGCGGAGGCGCAGCGCCCAGAGGAGGCGGGGCGCGCCCTGGCCGCGGTGCTGAGGAGCTGCGGCGACGCGAGCCGACCT
AlaArgMetGluGlyAlaGluAlaGlnArgProGluGluAlaGlyArgAlaLeuAlaAlaValLeuArgSerCysGlyAspAlaSerArgPro

559
GCACGCGGTGCAGGGCTGGGCTGCCCGGAGCTGGCTGCCGGCACGTTGTGAAACAGCTATTTTATTCCCAATGCGTTCCAAGAAGATTTTTGG
AlaArgGlyAlaGlyLeuGlyCysProGluLeuAlaAlaGlyArgLeuEnd

652
AAGCGTGCATCCAGTGAGACCTTTGAGGCTTGAGTCTTTTAGTGCCTGCATTTGGGTCAAAGCCACAGATGTATTAAACAAAACCATCCTGTT

745
TTCCTATGGCACAAAGAGGAATCCATATGAAATCCAGCTGTATCTCAGCTACCAATCCATAGTGTTTGTGGTGGGTGGAGAGGAGAACAAACT

838
GGTTGCTGAAGCCATGGTTTCCCTGGGAAGGTGGACCCACCTGTGCGGCACCTGGAATTCAGAGGAAGGGCTCACATCCTTGTGGGTAAATGG
```

FIG. 4B

931
TGAACTGGCGGCTACCACTGTTGAGATGGCCACAGGTCACATTGTTCCTGAGGGAGGAATCCTGCAGATTGGCCAAGAAAAGAATGGCTGCTG

1024
TGTGGGTGGTGGCTTTGATGAAACATTAGCCTTCTCTGGGAGACTCACAGGCTTCAATATCTGGGATAGTGTTCTTAGCAATGAAGAGATAAG

1117
AGAGACCGGAGGAGCAGAGTCTTGTCACATCCGGGGGAATATTGTTGGGTGGGGAGTCACAGAGATCCAGCCACATGGAGGAGCTCAGTATGT

1210
TTCATAAATGTTGTGAAACTCCACTTGAAGCCAAAGAAAGAAACTCACACTTAAAACACATGCCAGTTGGGAAGGTCTGAAAACTCAGTGCAT

1303
AATAGGAACACTTGAGACTAATGAAAGAGAGAGTTGAGACCAATCTTTATTTGTACTGGCCAAATACTGAATAAACAGTTGAAGGAAAGACAT

1396
TGGAAAAAGCTTTTGAGGATAATGTTACTAGACTTTATGCCATGGTGCTTTCAGTTTAATGCTGTGTCTCTGTCAGATAAACTCTCAAATAAT

1489
TAAAAAGGACTGTATTGTTGAACAGAGGGACAATTGTTTTACTTTTCTTTGGTTAATTTTGTTTTGGCCAGAGATGAATTTTACATTGGAAGA

1582
ATAACAAAATAAGATTTGTTGTCCATTGTTCATTGTTATTGGTATGTACCTTATTACAAAAAAAATGATGAAAACATATTTATACTACAAGGT

1675
GACTTAACAACTATAAATGTAGTTTATGTGTTATAATCGAATGTCACGTTTTTGAGAAGATAGTCATATAAGTTATATTGCAAAAGGGATTTG

1768
TATTAATTTAAGACTATTTTTGTAAAGCTCTACTGTAAATAAAATATTTTATAAAACTAAACGGAATTC 1837

FIG. 5

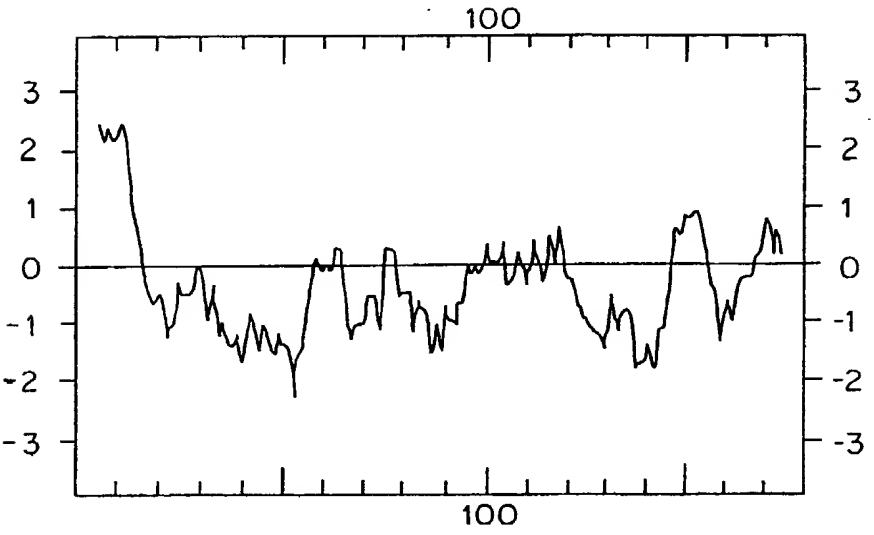
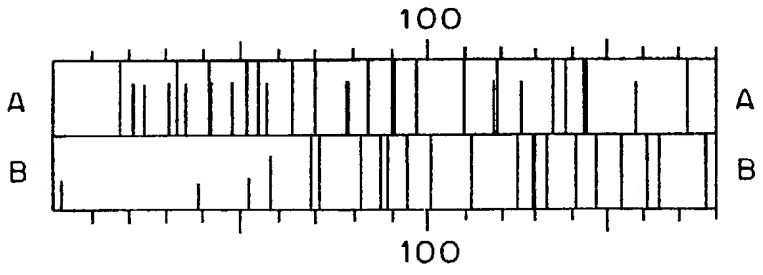


FIG. 6



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/00337

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07K 15/00,15/28; C12P 21/02; C07H 15/12; C12Q 1/68; G01N 22/53
US CL : 530/350,351,395,387; 435/69.1,69.5,6,7.1; 536/27

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	530/350,351,395,387; 435/69.1,69.5,6,7.1; 935/78,81; 536/27, 930/140/144

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

COMPUTER DATA-BASE SEARCH AND SEQUENCE SEARCH

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
x/y	Proceedings of the National Academy of Science, Vol. 86, issued April 1989, Lowenthal et al, "Tumor Necrosis Factor γ Induces Protiens that Bind Specifically to KB-Like Enhancer Elements and Regulate Interleukin 2 Receptor γ-Chain Gene Expression in Primary Human T Lymphocytes" see 2331, 2333-23334.	1/1
x/y	The Journal Biological Chemistry, Vol. 261, No. 21, issued 25 July 1986, Kirstein et al., "Tumor Necrosis Factor Induces Synthesis of Two Proteins in Human Fibroblasts", see pages 9565-66.	1/1
x/y	Molecular and Cellular biology, Vol. 10, No. 5, issued May 1990, Lee et al., "Isolation and Characterization of Eight Tumor Necrosis Factro-Induced Gene Sequences from Human Fibroblasts", see all.	1-17/4-28
x/y	The Journal Interferon Research, Vol. 9, supplemental 2, issued October 1989, Lee et al, "Eight TNF-Inducible cDNA Clones From Human FS-4 Fibroblasts: Regulation By Interferons, Cytokines and Growth Factors" see pages 5-145	1-17/4-28

* Special categories of cited documents:[16]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
06 MAY 1992	18 MAY 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	Deborah Frieda for Garnette D. Draper, Primary Examiner

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

x	Chemical Chemistry, Vol. 276, No. 11, issued 1981, Sevier et al., "Monoclonal Antibodies in Clinical Immunology" see all	18-22,26,28
X	U.S., A, 4,581,333 (Kourilsy et al.), 8 April 1986, see all	23-25,27

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

Please See Attached Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PREVIOUS SHEETS

VI. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, Claims 1-17 to the protein; DNA, host and method of making.
Group II, Claims 18-119 to antibodies.
Group III, Claims 20,22,26,28 to a diagnostic method using antibodies.
Group IV, Claims 23-25, 27 to hubridization assay.